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Photocycles of Bacteriorhodopsins Containing 13-Alkyl-Substituted Retinals†

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ABSTRACT: Three retinal analogues, carrying at position 13 a hydrogen atom, an ethyl group, or a *n*-propyl group instead of the naturally occurring methyl group, were incorporated into bacterioopsin. The absorption maxima of the newly formed analogue bacteriorhodopsins (BRs) are in the range of 545–565 nm; the positions of those maxima decrease with increasing length of the alkyl substituent. No light/dark adaptation was found for the analogue BRs. Flash photolytic experiments revealed the presence of separate cis and trans photocycles. Each of the analogue BRs forms, within several microseconds, an intermediate with a strongly blue-shifted absorption maximum, comparable to the M-intermediate of the trans photocycle of BR. The M-intermediates of the ethyl and the propyl derivative decay within a few milliseconds, as in BR, and their proton translocating ability correlates with the proportion of the all-trans isomer in the binding sites. The 13-demethylretinal BR (13-dm BR), however, is an exception. Although 15% of the retinal is in the all-trans isomeric state [Gärtner, W., Towner, P., Hopf, H., & Oesterhelt, D. (1983) *Biochemistry 22*, 2637–2644], in the binding site, maximally 3% of proton translocating activity was found. This discrepancy can be resolved if the delay time of about 20 ms of the M-intermediate in the trans photocycle of 13-dm BR is taken into account. The cis photocycle of 13-dm BR apparently consists of only one red-shifted intermediate absorbing around 610 nm and decaying with a half-time of 250 ms back to the initial state. At increasing irradiance of the monitoring light beam secondary photochemistry leads from this intermediate to an M-like state (λ_{max} about 420 nm), which has a half-life of 1.7 s.

The most abundant membrane protein of *Halobacterium halobium*, bacteriorhodopsin (BR),[1] acts as a light-driven proton pump that allows the halobacteria to grow phototrophically [for review see Stoeckenius and Bogomolni (1982) and Oesterhelt and Krippahl (1983)]. The isomeric state of retinal in bacteriorhodopsin can be either all-trans or 13-cis. These species equilibrate in the dark to yield a 1:1 ratio, whereas in the light-adapted form of the chromoprotein nearly

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[1] Abbreviations: BR, bacteriorhodopsin; BO, bacterioopsin; dm, demethyl; 13-Et, 13-ethyl; 13-Pr, 13-*n*-propyl; 13-dm BR, 13-demethylretinal-containing bacteriorhodopsin; OD, optical density; S/N, signal to noise ratio; I_{560}, intermediate absorbing at 560 nm.

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100% of the retinal is in the all-trans isomeric configuration (Oesterhelt et al., 1973; Pettei et al., 1977). The 13-cis and all-trans BR forms undergo different photocycles upon illumination [for review see Stoeckenius and Bogomolni (1982)]. Only the trans photocycle is connected to proton translocation (Fahr & Bamberg, 1982; Gärtner et al., 1983) and has been analyzed in great detail. The most important event in this photocycle is the reversible all-trans to 13-cis isomerization of the retinal molecule, as demonstrated by both resonance Raman spectroscopy (Aton et al., 1977) and chemical analysis (Tsuda et al., 1980). The necessity of a trans–cis isomerization for effective proton translocation was proven with the help of retinal analogues, whose structures were modified in order to prevent isomerization of the C13/14 double bond. In these studies configurationally and conformationally blocked molecules were inactive in proton translocation (Fang et al., 1983; Kölling et al., 1984). A detailed spectroscopic analysis of the conformationally blocked (9–12) phenylretinal showed no photochemical activity whatever, thus demonstrating that already the formation of the first intermediate, K, in the trans cycle involves bond rotations (Polland et al., 1984).

Less information is available about the intermediates of the cis cycle (Dencher et al., 1976). A specific feature of the cis cycle of BR is the high probability of retinal to switch into the trans cycle, explaining the almost exclusive presence of the all-trans isomer after light adaptation.

Again with the help of retinal analogues, it was shown that changed retinal–protein interaction can cause the opposite transition, i.e., a switch from the trans to the cis cycle. For example, α-retinal showed upon light adaptation an increase of the proportion of the 13-cis isomer in the binding site (Towner et al., 1980). The strongest effect on cis/trans equilibria, favoring the 13-cis isomer upon light adaptation as well as in the dark, was found when specifically the methyl group at C13 was replaced by a hydrogen atom (Gärtner et al., 1983; Tavan et al., 1985a,b). The absence of the methyl group at C13, but at no other position, leads to a predominant formation of the 13-cis isomer (85%) and to an almost negligible proton translocation activity of about 1–3%, even though the cis/trans ratio of 85:15 persists under illumination (Gärtner et al., 1983). This indicates that the remaining 15% of the pigment containing the all-trans isomer of 13-demethylretinal must be much less active in proton translocation than BR containing *all-trans*-retinal. Therefore, BR containing 13-dm-retinal was chosen for a detailed investigation of its photochemical properties. The results obtained with this analogue are compared with the corresponding methyl compound (retinal) and with 13-ethyl- (13-Et) and 13-*n*-propyl- (13-Pr) retinal (for structures see Figure 1) in order to examine the influence of the length of the substituent at that position on the photochemistry and transport function of BR. The results presented in this paper clearly demonstrate that the lack of an alkyl group at C13 not only favors the inactive 13-cis form of the analogue BR but also sufficiently lowers the frequency of the active all-trans photocycle to explain the very low transport activity of this chromoprotein.

Materials and Methods

Origin of Retinals and Preparation of Isomers. The synthesis of 13-dm-retinal and some of the spectral and biochemical properties of its reconstitution product upon reaction with bacterioopsin (BO) have been described already (Gärtner et al., 1980, 1983). 13-Ethyl- and 13-*n*-propylretinal were generous gifts of Dr. P. Ermann and Prof. H. J. Bestmann (University of Erlangen, FRG). Some of the interactions of these retinals with halobacterial proteins were recently described (Schimz et al., 1983). All retinals were checked for purity by thin-layer chromatography and separated into the isomers by HPLC after production of a photosteady state by irradiation in 2-propanol (Gärtner et al., 1980). The isolated isomers were identified by 400-MHz ^{1}H NMR.

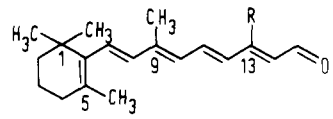

FIGURE 1: Structures of retinal analogues used for reconstitution reactions. R = H, 13-demethylretinal; R = CH_3, retinal; R = C_2H_5, 13-ethylretinal; R = C_3H_7, 13-*n*-propylretinal.

Formation and Characterization of Reconstituted BRs Containing Retinal Analogues. BRs were reconstituted by addition of the all-trans or the 13-cis isomers of the retinal analogue to a BO suspension in water in a molar ratio of 0.9:1. The formation of chromoproteins was monitored spectroscopically with an Aminco DW2 spectrophotometer in the wavelength range between 340 and 700 nm, with BO suspensions of the same concentration as reference. The samples containing fully reconstituted chromoproteins (free retinal no longer detectable by spectroscopy) were divided into two parts, which were either illuminated to detect any changes in the absorption maximum due to the formation of the light-adapted state (LA) or left in the dark [dark-adapted state (DA)]. Both samples were then extracted with organic solvents to determine the ratio of the 13-cis to the all-trans isomers in the binding site, as described earlier (Gärtner et al., 1983). The biological function of the reconstituted BRs containing the 13-alkyl analogues of retinal was tested in a cell vesicle system and measured as initial rate of light-induced proton translocation according to the methods of Gärtner et al. (1983) and Oesterhelt (1982). Preparations containing BO and reconstituted BR were taken as references; the activity of the latter sample was set as 100%.

Preparation of Samples Used for Flash Photolytic Experiments. BRs containing the four retinals were reconstituted as described and diluted to an optical density of 1.0 at their λ_{max} values. Samples were always freshly prepared and were analyzed in water or in 50 mM phosphate buffer (pH 7.0). The choice of water or phosphate buffer did not affect the kinetic behavior of the samples. No irreversible changes in absorption due to denaturation or photobleaching were detected in the samples.

Measurement of Light-Induced Absorbance Changes. The experimental setup used for the flash photolysis experiments is described in Vogel et al. (1983). The measuring light beam had a maximum intensity of 30 mW cm^{-2} at 620 nm and was varied by neutral glass and interference filters. The samples were flashed up to 4000 times with light from an excimer laser pumped dye laser at 20 ± 2 °C (λ_{ex} = 570 nm). The averaged signals were then transferred to a microcomputer (TI 9900) for further processing by the method described below.

Results

Biochemical Properties of BRs Containing Alkyl-Substituted Retinals. Three retinal derivatives modified in the substituent of C13 of retinal were used for the measurements reported here. A hydrogen atom (13-demethylretinal), an ethyl group (13-ethylretinal), or a *n*-propyl group (13-*n*-propylretinal) replaced the 13-methyl group of the retinal molecule itself (Figure 1). Some of the properties of these retinal analogues and their reaction products with BO are compared with those of retinal and of BR in Table I. The absorption maxima of the free retinal analogues are uniformly

Table I: Spectral and Functional Properties of Retinal Analogues Substituted at C13

compd	λ_{max} (nm) of free aldehyde (all-trans isomer) in 2-propanol	λ_{max} (nm) of analogue BR (LA/DA)[a]	ratios of all-trans:13-cis isomers		activity[b] of analogue BRs (% of retinal)
			LA[a]	DA[a]	
13-demethylretinal	380	565/565	15:85	15:85	1–3
retinal	380	570/558	91:9	57:43	100
13-ethylretinal	379	559[c]	67:33	67:33	70
13-*n*-propylretinal	380	545[c]	50:50	50:50	40

[a] LA/DA: Light- and dark-adapted states; for retinal a λ_{max} value of 548 nm of the pure 13-cis isomer is known. Using the difference of 22 nm between the 13-cis and the all-trans forms for a linear extrapolation, the λ_{max} values of the pure isomeric forms of the other retinal analogues can be estimated. [b] Activity was determined as initial rates of light-induced proton translocation in cell vesicles after reconstitution of the chromoproteins. [c] The value corresponds to a reconstituted sample in diffuse light. Illumination (5 min, >515 nm) causes a slight decrease in optical density and a λ_{max} of 556 nm for 13-ethylretinal and a λ_{max} value of 537 nm for 13-*n*-propylretinal.

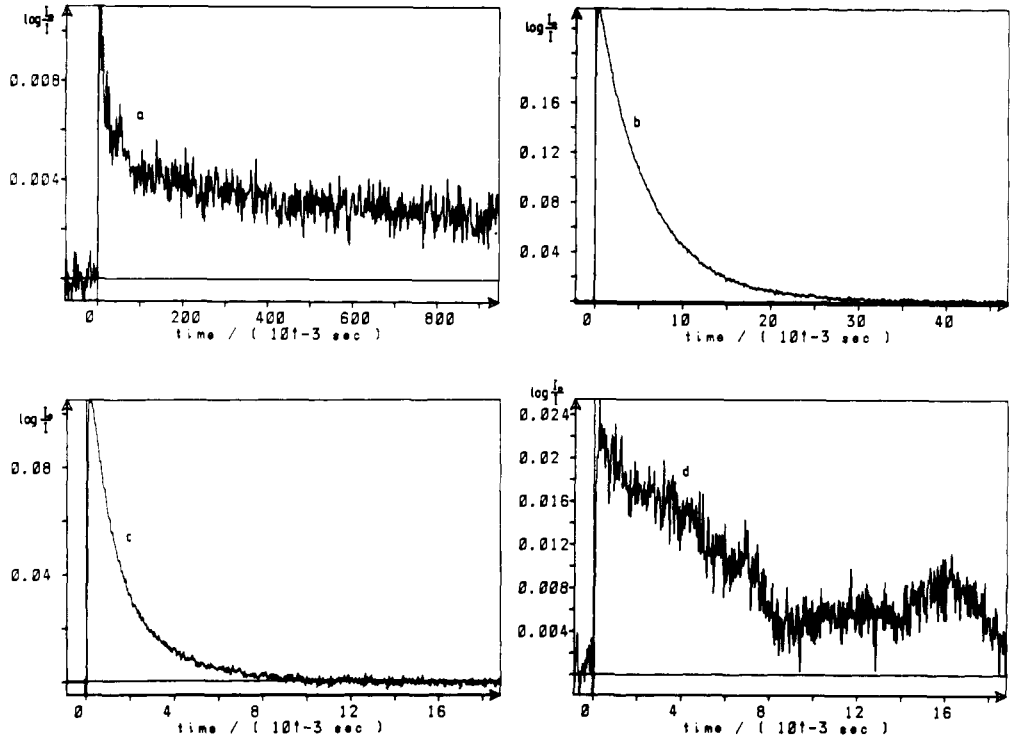

FIGURE 2: Laser flash induced absorption changes as log (I_0/I) (λ_{ex} = 570 nm) of the various chromoproteins. I_0 defines the amount of light measured after it passes through the cuvette without a flash illumination; I defines the corresponding value after flash illumination. The monitoring wavelength was 420 nm for 13-dm BR (a), 400 nm for BR (b), 420 nm for 13-Et BR (c), and 410 nm for 13-Pr BR (d). Note the different time scales.

close to 380 nm, indicating that the alkyl substituent has little influence on the π-electron system nor does it lead to steric hindrances in organic solvent solution. All compounds reconstituted chromoproteins upon association with BO. The absorption maxima of the chromoproteins depend on the length of the alkyl substituent; if extrapolated to the pure isomeric forms, the absorption maxima can be arranged in a series of R = H > CH_3 > C_2H_5 > C_3H_7, with R = H (13-dm-retinal) having the most red-shifted absorption maximum.

The isomeric composition of the retinals in the dark-adapted state (DA) of the BRs was between 50% and 70% all-trans for retinal, 13-Et-retinal, and 13-Pr-retinal. In contrast to this, only 15% of the 13-dm-retinal is present in the all-trans configuration. Upon light adaptation, retinal changes its isomeric distribution in BR to nearly 100% all-trans [Table I lists 91%, found by Gärtner et al. (1983) but see also Tsuda et al. (1980)], whereas the other compounds remain essentially at their DA ratios. According to the results of Fahr and Bamberg (1982), the proton translocation activity of BRs containing various retinal analogues should correlate with the percentage of the trans isomer present in the light-adapted state. Indeed, we find that the activities of the ethyl and propyl derivatives agree, within the errors of determination, with this prediction. In contrast, the transport activity of 13-dm BR is 5-fold lower than expected, indicating that the trans cycle of this compound must have unusual features with slow proton translocation [cf. Gärtner et al. (1983)].

Photochemical Behavior of Analogue BRs. The pulse duration of the laser used for flash photolytic experiments was 10–15 ns, and the time resolution of the transient digitizer restricted the detection of intermediates to those with half-times longer than 1 μs. Thus, the instrumental setup is sufficient to study the decay of the L-intermediate and concomitant rise of the M-intermediate. The M-intermediate is especially suited to analyze the photoreactions of BR and its analogues for contributions by the all-trans cycle, because in the description of the photocycles of BR published so far, no intermediate with a comparable blue-shifted absorption maximum and a rise time in the microsecond range has been reported for the cis cycle (Dencher et al., 1976; Sperling et al., 1977). Furthermore, the absorption band of M does not overlap significantly with any other intermediate of the trans cycle. Thus, one can expect that the quantitation of the M-intermediate might allow an estimation of the proportion

Table II: Temporal Changes of the Optical Density around 400 nm for Various Retinal Analogue BRs

compd	monitoring wavelength (nm)	increase to half-height (μs)	decrease to half-height (ms)	max OD change (log I_0/I)
13-dm BR	420	25/400	20/1700	0.011[a]
BR	400	50	4.5	0.240
13-Et BR	420	20	1.2	0.110
13-Pr BR	410	*b*	5	0.024

[a] This signal consists of two components with a ratio of about 1.5:1. [b] Not detectable for instrumental reasons (see text).

of the trans cycle to the total photochemical activity of BR. Figure 2 shows examples of original traces of the absorption changes around 400 nm after flash illumination of the chromoproteins 13-dm BR, BR, 13-Et BR, and 13-Pr BR with actinic light of 570 nm. The most conspicuous feature in Figure 2 is that the decay times of the M-intermediates of BR (b), 13-Et BR (c), and 13-Pr BR (d) are similar but that the M-intermediate of 13-dm BR (a) clearly decays in a biexponential way and much slower.

The rise times of the various M-intermediates are between 20 and 50 μs and are listed in Table II as the times required to reach half-maximal change in OD after the laser flash at the indicated wavelength. For 13-Pr BR no rise time could be measured exactly, because the signal to noise ratio (S/N ratio) was too low at the monitored wavelength. Measurements at wavelengths below 400 nm, which would be closer to the maximal absorbance changes of the M-intermediate of 13-Pr BR, could not be performed for experimental reasons. Because the decay is monoexponential (except for 13-dm BR), the decay times are again given as the times necessary to reduce the OD change to half its maximal value, whereas for 13-dm BR an automatic fitting procedure allowed us to determine the two decay times (see Table II). For the M-intermediates of BR and 13-Pr BR similar half-times are found, but the intermediate of 13-Et BR decays faster with about 1.2 ms. The exception is 13-dm BR, which decays in two phases, one component with 20 ms, i.e., 5 times slower than BR, and a second very slow component with 1.7 s. This phenomenon will be described in more detail below.

Photocycle of 13-dm BR. The photoinduced absorption changes of 13-dm BR were recorded at 14 different wavelengths between 400 and 680 nm and on four different time scales ranging from 1 μs to 10 s. Owing to the different magnitudes of the optical absorption changes at the various wavelengths, the traces show very different S/N ratios. Figure 3 illustrates the quality of the recorded optical density changes (λ_{obsd} = 600 nm) and gives a demonstration of how the data were evaluated. The first step consists of a semilogarithmic plotting of the trace recorded on the longest time scale (Figure 3a, trace 1). This immediately yields the smallest rate constant (longest decay time) and, after extrapolation to t = 0, the corresponding amplitude. These two parameters were used to subtract the corresponding exponential (fit function) from the experimental curve (trace 1) in Figure 3a to yield a "reduced" curve (trace 2 in Figure 3a), which contains information on the decay with the second smallest rate. Trace 1 in Figure 3b represents the absorbance change recorded on the next faster time scale. A reduced function, trace 2 in Figure 3b, is again generated by subtraction of the exponential function whose parameters were determined as described above. It is identical with trace 2 of Figure 3a, except that the higher time resolution makes available more data points for the evaluation of the time constants. If the complexity of the kinetic process shown in Figure 3 is considered, it becomes

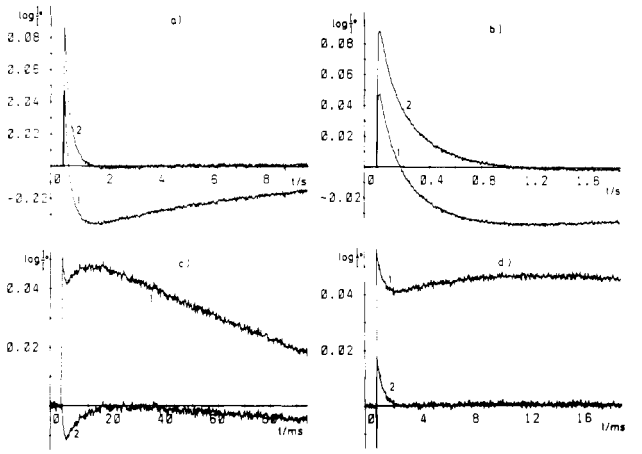

FIGURE 3: Laser flash induced absorption changes (λ_{ex} = 570 nm) of 13-dm BR monitored at λ_{obsd} = 600 nm with different time resolutions: (a) 1 s/division; (b) 200 ms/division; (c) 10 ms/division; (d) 2 ms/division. Curves 2 were derived after subtraction of the calculated (multi-) exponential fit curves from the experimental curves (1); for details see text.

quite obvious that this somewhat complicated evaluation procedure has to be performed to derive trustable data over the entire time range. With the described procedure the exponential function with the next higher time constant is then derived. Subtraction of the first two exponential functions from trace 1 of Figure 3c, which is recorded on the next faster time scale, yielded the next reduced function (trace 2 in Figure 3c), which then was treated as described until the limit in time resolution of the apparatus was reached or no further fits could be performed (Figure 3d). This computation was carried out for each measured wavelength. The half-times and the amplitudes computed were grouped and the following results obtained.

The shortest detectable half-times of about 4 (limit of time resolution) and 25 μs are connected to the rise in absorption around 610 and 420 nm. The two intermediates corresponding to these changes are designated I_{610} and M_{420}. They cannot be formed sequentially but must be formed via alternative routes because no decreasing exponential function around 610 nm with a time constant of 25 μs was found. We assigned these intermediates, on the basis of the relative magnitudes of their absorption changes, to the trans and cis cycles, respectively (see Figure 5). The next prominent change of absorption occurred as a decrease around 610 nm and a further increase around 420 nm with a half-time of 400 μs. Finally, the decrease of the absorption change around 420 nm occurs with half-times of 20 ms and 1.7 s, whereas the absorption changes at 610 nm are reversed with a time constant of 250 ms. Besides these major absorption changes, minor changes occur around 430, 460, and 500 nm. These changes were found to be produced by secondary photochemical processes, induced by high measuring light intensity and not analyzed further. Similarly, the major absorption changes can be influenced by the intensity of the measuring light beam as shown by the experiment of Figure 4. Time-dependent absorbance changes at 620 nm upon a flash were measured at two different intensities of the measuring beam. Trace a was registered when the intensity was reduced to a minimum of 20 μW/cm^2, whereas trace b reflects the increase of intensity to a maximum of 30 mW/cm^2. It becomes immediately obvious that the time constant of 250 ms dominates trace a and that in trace b the amplitude decreases dramatically with a change in sign and changes in the rate constants. The same experiment carried out at a measuring wavelength of 420 nm revealed an increase

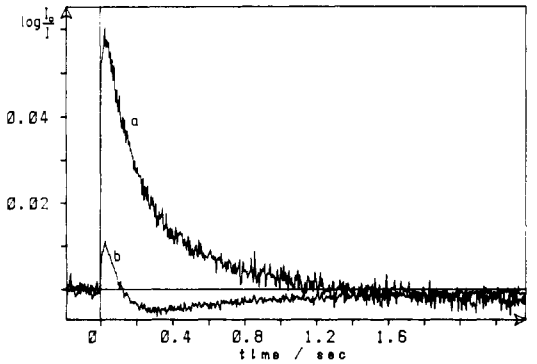

FIGURE 4: Laser flash induced absorption changes (λ_{ex} = 570 nm) of 13-dm BR monitored at λ_{obsd} = 620 nm. (Curve a) Intensity of monitoring light 20 μW/cm^2. (Curve b) Intensity of monitoring light 30 mW/cm^2.

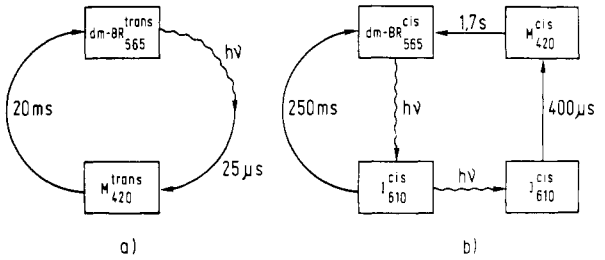

FIGURE 5: Proposal for the photocycles of 13-dm BR.

in the amplitude of the slow component decaying with 1.7-s half-time with increasing irradiance (traces not shown). Figure 5 summarizes the result of all measurements. All-trans dm BR (15%) forms an M_{420} intermediate (rise time 25 μs) that decays with a half-time of 20 ms to the initial state. 13-cis 13-dm BR (85%) forms I_{610} (rise time <4 μs) that has a long lifetime of 250 ms. At very low irradiance of the measuring beam (20 μW/cm^2) only these two reactions contribute to the absorption changes. When the irradiance is increased to values used typically in flash photolysis, no changes in the trans part of the 13-dm BR photochemistry are observed. Neither M^{trans}_{420} accumulates in the photostationary state to a measurable extent because of its decay time of 20 ms, nor could it absorb light from the laser flash (λ_{ex} 570 nm) potentially causing secondary photoproducts. In contrast, I^{cis}_{610}, due to its slow decay time of 250 ms, accumulates in the measuring beam to a photostationary-state concentration high enough to form the substrate for secondary photochemistry. The laser flash at λ_{ex} 570 then is absorbed not only by dm BR^{cis}_{565} and produces more I^{cis}_{610} but also by I^{cis}_{610} already produced by the measuring light. As a consequence, J^{cis}_{610} is formed followed by M^{cis}_{420}, which has a rise time of 400 μs. This intermediate decays very slowly (1.7 s) back to dm BR^{cis}_{565}, explaining the biphasic decay at 420 nm in Figure 1a and the drastic change in transient absorption changes at 620 nm upon changes in measuring light intensity.

Figure 6 compares on a logarithmic time scale the course of absorbance changes at 620 nm of 13-dm BR performed under low irradiance with that of BR. In trace b the L → M and M → BR_{570} transitions of BR are resolved and the parameters obtained by automatic fitting satisfy the kinetic constants known for the photocycle of BR (Lozier et al., 1975; Maurer et al., 1987a,b). The same fitting procedure applied to trace a (13-dm BR) yields time constants of about 400 μs and 20 ms for the depression in the plateau of the trace and 250 ms for its decay. These time constants justify the scheme of the two photocycles of Figure 5 and are attributed to the reformation of trans-dm BR (20 ms), the decay of the photoproduct of 13-cis-dm BR (I^{cis}_{610}, 250 ms), and the conversion of the secondary photoproduct J^{cis}_{610} to M^{cis}_{420} (400 μs).

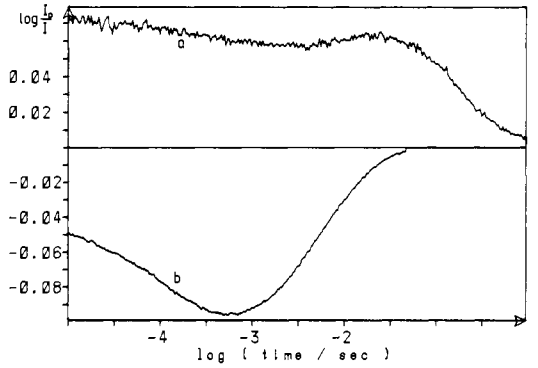

FIGURE 6: Laser flash induced changes in optical density (λ_{ex} = 570 nm) of BR (a) and of 13-dm BR (b), detected at 620 nm, shown on a logarithmic time scale. Time is given in seconds. Frequency of flashes $^1/_{20}$ s. The intensity of the measuring light beam was 20 μW/cm^2.

Discussion

The different activities of 13-alkyl-substituted retinals in bacteriorhodopsin reflect the different all-trans isomer content of their chromophores. Only 13-dm BR is an exception, and the explanation is based on the trans cycle of 13-dm BR, which is slower by a factor of 4–5 compared with that of retinal. This is in agreement with theoretical calculations predicting an increased energy barrier for thermal reisomerization of the C13/14 bond (Tavan et al., 1985a).

The 13-cis 13-dm BR after excitation has two possibilities to return to the initial state: (a) the thermal reaction back to BR_{565} from I^{cis}_{610} with a half-time of 250 ms or (b) entering by a second excitation the route via M^{cis}_{420} with a half-time for reformation of BR_{565} within 1.7 s. As shown in Figure 4, trace b, an irradiance of 30 mW/cm^2 is already enough to produce appreciable steady-state concentrations of I^{cis}_{610} and therefore opens the route via M^{cis}_{420}. Stationary-state concentrations of intermediates are approximately inversely proportional to their thermal decay constants and increase with irradiance. Thus, in practice of continuous illumination experiments bacteriorhodopsin with a relaxation time of 50 μs (transition of L → M) is not subjected to appreciable secondary photochemistry at neutral pH but molecules like 13-dm BR are.

If white light were used for monitoring absorption changes (monochromatizing the light after passing through the sample), M-states absorbing around 400 nm could also produce photoproducts. For BR, no complications would arise because M decays fast (5 ms). M^{cis}_{420} in 13-dm BR, however, decays slowly (1.7 s) and would be a substrate for photochemical excitation even under low (<10 mW/cm^2) irradiance. Examples of how nature uses this photochromism coupled to variation of thermal relaxation constants are sensorrhodopsin and halorhodopsin in halobacteria (Spudich & Bogomolni, 1984; Hegemann et al., 1985).

Because the trans and cis cycles in 13-dm BR equilibrate thermally or photochemically, molecules from the trans cycle can be trapped in M^{cis}_{420} of the inactive 13-cis cycle upon continuous illumination, i.e., during the proton translocation assay. Indeed, the dependence of proton translocation activity on irradiance of 13-dm BR is very different from that of BR (Gärtner et al., 1983).

The long lifetime of K^{cis} in 13-cis BR (37 ms; Sperling et al., 1977) and I^{cis}_{610} in 13-dm BR compared to the K^{trans} species has been explained by a different environment of 13-*cis*-retinal

and its photoproduct made from anionic groups in various protonation states (Trissl & Gärtner, 1987). It is interesting to note that the factor of about 6 for the decay times of K^{cis} in BR and I^{cis}_{610} in 13-dm BR is similar to that for M decay in the respective trans cycles, reflecting in both cases a similar influence of the lack of the 13-methyl group on the stereodynamics of retinal.

Nothing is known about the photochemical reaction of the 13-*cis*,15-*syn*-retinal (dark-adapted) configuration in BR, but one of the following intermediates can convert into the 13-*trans*,15-*anti*-retinal (light-adapted) configuration. Since this double isomerization also occurs in the dark but takes about 30 min at room temperature (Oesterhelt et al., 1973), the photochemical reaction of 13-cis,15-syn should involve isomerization reactions either around the 13–14 or around the 15–16 bond but not simultaneously (Orlandi & Schulten, 1979). The question then remains about the nature of the photochemical reaction of I^{cis}_{610} that leads to M^{cis}_{420} which is distinct from M^{trans}_{420} by its long lifetime and should not be an all-trans state. Possibly this state involves an 11-cis configuration since 13-dm retinal is so far the only retinal structure that can adapt an 11-cis configuration in the binding site of bacterioopsin (Gärtner et al., 1983). The slow return of M^{cis}_{420} proposes that the deprotonated Schiff base encounters a slightly different amino acid environment, preventing efficient reprotonation as prerequisite to thermal cis–trans isomerization (Tavan et al., 1985a). The detailed analysis of 13-dm BR molecules containing appropriately deuteriated retinals by resonance Raman spectroscopy will provide more insight into the stereodynamics of the retinal molecule in the binding site.

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