home-built spectrometer[10] at 38.8 MHz. Electron micrographs were also determined immediately by negative staining with 2% ammonium molybdate on 200 mesh Formvar carbon-coated grids in a Philips 300 electron microscope at 80 kV.

Figure 1 contains ^2H NMR spectra of various selectively deuterated palmitic acids in egg phosphatidylcholine/sphingomyelin vesicles. The widths of the peaks at half height $\Delta\nu_{1/2}$ were (top to bottom) [2,2-$^2H_2$] 386, [4,4-$^2H_2$] 446, [5,5,6,6-$^2H_4$] 612, and [11,11,12,12-$^2H_4$] 365 Hz. These widths, of course, would be heavily weighted to reflect the contributions due to the smaller vesicles. Lest the addition of 15% sphingomyelin be suspected of drastically decreasing the bilayer order, the spectrum of ≈5 mol % [5,5,6,6-$^2H_4$]palmitic acid in egg phosphatidylcholine vesicles has been determined and gave a signal with a $\Delta\nu_{1/2}$ = 520 Hz.

Vesicles are heterogeneous particles with diameters from <250 to >600 Å, hence, the ^2H NMR line shapes in Figure 1 are the superposition of resonances of large to small sizes. We have determined a statistical weighting for six size categories on the basis of vesicle sizes determined from electron micrographs.[11] A theoretical line shape $S(\nu)$ was calculated on the basis of a sum of Lorenztian lines

$$S(\nu) = A\sum_{i=1}^{n} \frac{F_i\bar{R}_i^2}{W_i} \frac{1}{1 + (4\nu^2/W_i^2)} \quad (1)$$

where F_i = fraction of vesicles in size category i,[11] and where $W_i \approx (9\pi/20)(e^2qQ/h)^2S_{CD}^2\tau_{v_i}$, (e^2qQ/h) is the static quadrupolar coupling constant (≈ 168 kHz[12]), and τ_{v_i} is the effective correlation time for the i^{th} set of vesicle reorientations given by

$$\tau_{v_i}^{-1} = \tau_{t_i}^{-1} + \tau_{d_i}^{-1} \quad (2)$$

$\tau_{t_i} = 4\pi\eta\bar{R}_i^3/(3kT)$ is the Stokes–Einstein term with η = solvent viscosity, $\bar{R}_i$ = mean radius of i^{th} vesicle set, k = Boltzmann's constant, and T = absolute temperature. The correlation time τ_{d_i} is given by $\tau_{d_i} = \bar{R}_i^2/(6D)$ where D is the diffusion coefficient of the palmitic acid in the bilayer plane. The diffusion coefficient has been measured for [$^2H_{31}$]palmitic acid in egg phosphatidylcholine vesicles, from the ^2H NMR, by the method outlined by Cullis.[13] The value we measured was $D \approx (7 \pm 1) \times 10^{-8}$ cm^2 s^{-1}.[14] The term $\bar{R}_i^2$ in eq 1 compensates for the fact that larger vesicles have greater numbers of palmitic acid molecules presumably proportional to the surface area of the vesicle, $4\pi R^2/S$, where S is the cross sectional area of a palmitic acid molecule. For convenience, S, along with other constants, is incorporated into the scaling factor A. Finally, the quantity S_{CD} is the C–D order parameter $S_{CD} = \langle(3\cos^2\theta) - 1\rangle/2$ where θ is the angle between the C–D bond and the normal to the bilayer surface and the angular brackets represent the average over all orientations of the lipid molecule.[15] (We have also assumed that the order is the same throughout the range of vesicle sizes.) Equation 1 will give an upper limit only to $|S_{CD}|$ since no contribution from local segmental motions of the chains is included.

We have used the solvent viscosity in our calculations, although, for a 15% solution, solution viscosity η = 1.85 cP might be more appropriate.[16] The resulting visual best fit simulated $S(\nu)$ is also shown in Figure 1 (dotted lines) for each chain segment and the calculated best fit order parameters (to the nearest 0.01) were S_{CD} = 0.08 for [2,2-$^2H_2$], 0.09 for [4,4-$^2H_2$], 0.10 for [5,5,6,6-$^2H_4$], and 0.07 for [11,11,12,12-$^2H_4$]. The spectrum for the terminal [16,16,16-$^2H_3$]palmitic acid, not shown in Figure 1, gave S_{CD} = 0.015. The value of S_{CD} from positions 2 to 12 of selectively deuterated stearic acid in egg phosphatidylcholine is 0.205 ± 0.035 at 30 °C,[2] similar to the values found in perdeuterated chains of dipalmitoylphosphatidylcholine.[17,18] A simulated spectrum with S_{CD} = 0.20 is also shown in Figure 1 for the 2-position and is clearly much too broad for the spectral line.

Our results with selectively deuterated palmitic acids are most directly compared with the pioneering work of Stockton et al.,[2] who studied selectively deuterated stearic acids in egg phosphatidylcholine vesicles at 30 °C. Those authors report ^2H NMR line widths of 1.2–1.3 kHz (giving S_{CD} = 0.23–0.24) for positions 2–10 and 800 Hz for position 12 (S_{CD} = 0.17), significantly larger than the line widths reported in this communication. We believe a substantial part of the disparity may lie in the values of τ_{v_i} in eq 1. Stockton et al.[2] prepared their vesicles in a bath-type sonicator for ≈1 h. This could lead to vesicles with substantially greater radii than used in their calculations, and, indeed, the authors admit to a possible disparity in their t_v of 40% (ref 2, p 964).

We have considered the possibility that structures smaller than vesicles (e.g., micelles containing palmitic acid) were formed and would contribute to the narrow portion of the NMR signals in Figure 1. First, [^{14}C]palmitic acid was included in an NMR run with [5,5,6,6-$^2H_4$]palmitic acid. The sample was subsequently chromatographed on Sepharose 4B at 4 °C. All of the radioactivity (92.5% of the initial counts) was eluted coincident with the OD 300-nm peak. No other radioactive peak was subsequently eluted, indicating the absence of smaller structures. Second, a synthetic phosphatidylcholine containing [4,4-$^2H_2$]palmitic acid at the *sn*-2 position was run in dipalmitoylphosphatidylcholine vesicles at 48 °C and gave a ^2H NMR signal whose width at half-height $\Delta\nu_{1/2}$ was 548 Hz.

We therefore conclude that acyl chain order in unilamellar vesicles is at least 2 times lower than in multilamellar liposomes.

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Registry No. Palmitic acid, 57-10-3; [2,2-$^2H_2$]palmitic acid, 62689-96-7; [4,4-$^2H_2$]palmitic acid, 30719-28-9; [5,5,6,6-$^2H_4$]palmitic acid, 75736-47-9; [11,11,12,12-$^2H_4$]palmitic acid, 75736-57-1.

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On the Binding Site of Bacteriorhodopsin. A Study with Artificial Pigments

Mordechai Sheves,* Timor Baasov, and Noga Friedman

Department of Organic Chemistry
Weizmann Institute of Science
Rehovot 76100, Israel

Michael Ottolenghi,* Rosalind Feinmann-Weinberg, and Varda Rosenbach

Department of Physical Chemistry
The Hebrew University, Jerusalem 91904, Israel

Benjamin Ehrenberg

Department of Physics, Bar-Ilan University
Ramat-Gan, Israel

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The molecular mechanism of the photocycle of bacteriorhodopsin (bR), the protein pigment in the purple membrane of *Halobacterium halobium*, has been the subject of considerable interest.[1] Artificial pigments, in which the native retinal moiety

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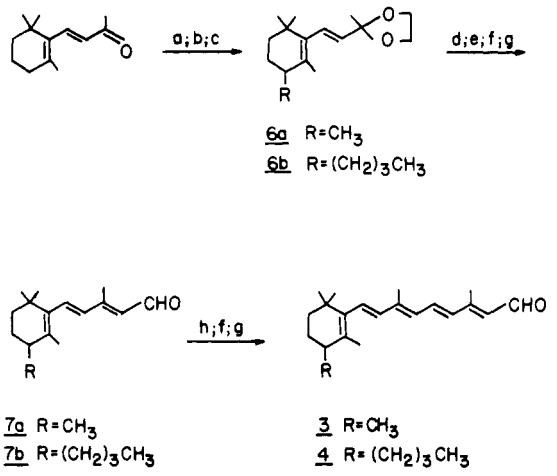
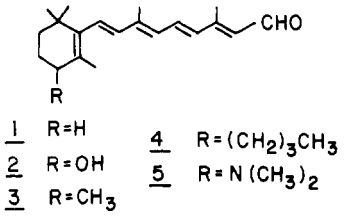
Scheme I[a]



[a] (a) $HOCH_2CH_2OH$/*p*-TsOH/benzene, reflux, 12 h; (b) NBS/CCl_4, reflux, 30 min; (c) CH_3Li for **6a** or BuLi for **6b**/THF, −78 °C, 1 h; (d) HCl/MeOH, 0 °C, 30 min; (e) $(EtO)_2POCH_2CO_2Et$/NaH/THF, room temperature, 30 min; (f) Dibal/ether, −78 °C, 1 h; (g) MnO_2/CH_2Cl_2, room temperature, 5 h; (h) $(EtO)_2POCH_2C(CH_3)=CHCO_2Et$/NaH/THF, room temperature, 1 h.

is substituted by synthetic analogues,[2] have provided a valuable tool in the study of the binding site and the photocycle of bacteriorhodopsin. It was recently shown that the spectra and photocycles of bR pigments are sensitive to modifications in the ring region of the molecule.[3] In the present work a systematic study of the effects caused by introducing steric hindrance in the vicinity of the ring is carried out. We have prepared modified retinals substituted at the C_4 ring position with groups bearing different bulkiness. The pigments based on chromophores **2**, **3**, **4**, and **5** were prepared by reconstruction with bacterioopsin. The



influence of the steric hindrance introduced on the spectrum and the photocycle was studied. The observations bear upon the retinal–opsin interaction in the binding site and on the molecular mechanism of the photocycle.

Compounds **3**[4] and **4** were synthesized using β-ionone as starting material (Scheme I). 4-(Dimethylamino)retinal (**5**) was synthesized as previously described.[5]

The absorption spectra of the pigments obtained by reconstituting bacterioopsin with the 4-hydroxy[6,7] (**2**) 4-methyl (**3**), 4-*n*-butyl (**4**), and 4-dimethylamino (**5**) analogues of retinal (denoted

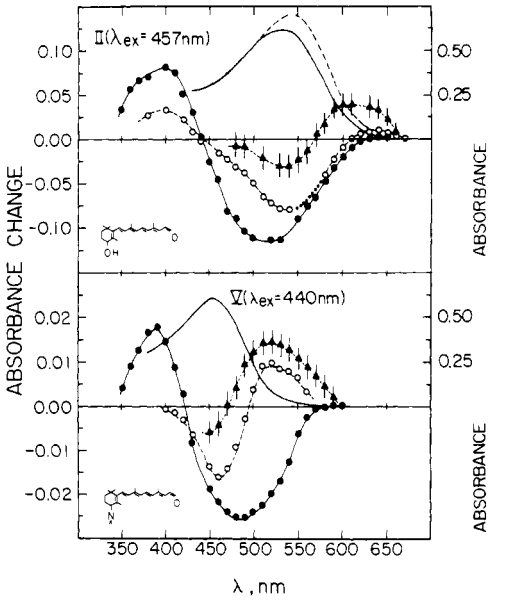


Figure 1. Absorption spectra and difference spectra in laser photolysis of pigments II and V: (—) dark adapted, (---) light adapted. Time after laser pulse: (▲) 1 μs, (○) 10 μs, (●) 1 ms.

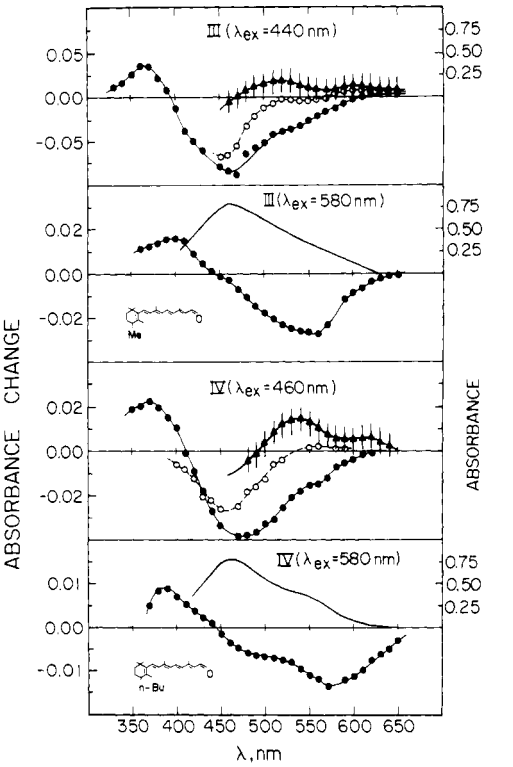


Figure 2. Absorption spectra and excitation wavelength effect of photolysis pigments III and IV: (—) absorption spectra of dark adapted. Difference spectra recorded the pulse: (▲) 1 μs (stage of K), (○) 10 μs, (●) 1 ms (stage of M).

as II, III, IV, and V, respectively) are shown in Figure 1 and 2, which are indicative of a systematic change in the spectrum in the order of II–V. In the spectra of III and IV the main absorption band is drastically blue shifted to 465 nm. However, a less pronounced shoulder reminiscent of the red bands of I and II is clearly evident around 550 nm. The 4-dimethylamino derivative V, shows only the single blue band at 455 nm.

Competition on opsin binding between *all-trans*-retinal **1** and **3–5** indicate that the synthetic analogues occupy the same binding site as *all-trans*-retinal. Light and dark adaptation phenomena in II are basically similar to those characteristic of native bR (I). Comparable effects could not be observed in pigments III–V. To

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(8) The chromophores were purified by HPLC before reconstitution. The reconstitution was done at pH 7.0 using Hepes buffer.

ascertain that the pigments are linked to the protein through a protonated Schiff base, we examined their resonance Raman spectra.[9] These measurements clearly reveal the contribution of C=NH$^+$ frequency to the spectra (1640 cm^{-1}), which shifted to lower frequency (1620 cm^{-1}), upon deuteration.[10] These observations confirmed the protonation of the Schiff base linkage.[11]

The observed spectral changes of the pigments may be correlated with an increase in the bulkiness of the C_4-substituent. Moreover, it is evident that the changes in going from I to V are basically due to a gradual reduction in the intensity of the red band and to a parallel increase in that of the blue band. The observations are indicative of two pigment species, characterized by different retinal–opsin interactions.

Pigments II–V were exposed to pulsed-laser photolysis. In all cases we observed an initial rise in absorption in the red, characteristic of the short-lived K intermediate of bR and a long-lived M species absorbing around 390 nm. In variance with II, V, and native bR, the photocycles of III and IV were markedly dependent on the excitation wavelength. Thus, excitation at 580 nm (in the range of the red band) led to a photocycle characteristic of a single pigment as in the case of native bR and II. However, excitation at 440 nm (in the range of the blue band) led to a K stage with two maxima in the red (520 ± 20 and 620 ± 20 nm). The formation of M was accompanied by two distinct depletions, corresponding to the red and to the blue absorption bands, respectively. It is thus evident that III and IV each consist of two species exhibiting two independent photocycles. The photocycle originating from the red absorbing species is comparable to those of I and II. The photocycle of the blue species is analogous to that of V.

The evidence presented above indicates that the retinal moiety may adopt at least two conformations while occupying the opsin binding site. Only one conformation is observed in bR (I), in which the red absorption band is indicative of a close proximity between the ring and an opsin charge as suggested by Nakanishi and Honig.[12] A second conformation, favored by increasing steric hindrance at the region of C_4, is associated with a drastic blue shift in absorption. The effect is attributed to a substantial increase in the separation between the polyene system and the "ring opsin charge". The blue absorbing species constitutes an interesting example of a bR pigment that retains the basic retinal chromophore system; however, this pigment's absorption is close to that of the corresponding free protonated Schiff base in solution (λ_{max} = 460 ± 20 nm), which obviously lacks the neighboring ring charge. As such it provides an additional direct support to the point charge model for bacteriorhodopsin.

The spectral shift, $\Delta\nu$(bR/K), associated with the generation of K, is of the order of 2500 cm^{-1} and is essentially independent of pigment and conformer. This observation strongly supports the claim that the primary photochemical event in bR is due to a charge separation associated with the protonated Schiff base and its opsin counterion and does not involve any other charges. While the ring charge appears to be dominant in controlling the spectra of the pigments and their K intermediates, its effect on the spectrum of M (characterized by a nonprotonated Schiff base) are relatively small. Thus, all M species of I–V absorb in the range between 380 and 410 nm, exhibiting considerable red shifts, relative to the corresponding nonprotonated Schiff bases in solution (λ_{max} = 350 nm).[13] The exact cause for the spectral shift in M is still under investigation.

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Registry No. 1, 116-31-4; **3**, 89196-58-7; **4**, 89196-59-8; **5**, 86948-78-9; **6a**, 89196-60-1; **6b**, 89210-15-1; **7a**, 89196-61-2; **7b**, 89196-62-3; $(EtO)_2POCH_2C(CH_3)$=$CHCO_2Et$, 39760-56-0; β-ionone, 79-77-6.

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Electron-Transfer Chemistry of the 20-Electron Complex $(C_6Me_6)_2Fe^0$ and Its Strategic Role in C–H Bond Activation and C–C Bond Formation[1]

Alex M. Madonik and Didier Astruc*

Laboratoire de Chimie des Organométalliques
ERA CNRS No. 477, Université de Rennes
35042 Rennes Cedex, France
Received December 28, 1983

Complexation of arenes in the 18-electron complexes (arene)$Cr(CO)_3$ and (arene)Fe^+Cp has provided specific synthetic applications because electron withdrawal by the transition-metal unit renders the arene ligand susceptible to nucleophilic attack or deprotonation.[2] We wish to describe here a different type of synthetically useful activation that results from the electron-transfer chemistry of the stable 20-electron complex $(C_6Me_6)_2Fe^0$,[3] a strong neutral reducing agent now available on a large scale.[4] This new approach is of particular significance since most of the classical, nonradical chemistry of other arene–transition-metal complexes fails with bis(arene)iron dications. For instance, it would have been desirable to make C–C bonds by nucleophilic attack of carbanions on $(C_6R_6)_2Fe^{2+}$ (R = H, Me) or to effect deprotonation ($\mathbf{1}^{2+}$, R = Me) by anionic O or N bases.[5] However, C, O, and N anions react with $\mathbf{1}^{2+}(PF_6^-)_2$ at −80 °C by electron transfer, providing mainly the purple 19-electron complex $\mathbf{1}^+PF_6^-$, characterized by Mössbauer spectra[4] of the reaction mixtures after slow warm up and removal of solvents (Scheme I).

$(C_6H_6)_2Fe^{2+}(PF_6^-)_2$ (**4**), and $(C_6Me_6)(C_6H_6)Fe^{2+}(PF_6^-)_2$ (**5**), react similarly.[6] We find that the desired, synthetically useful processes can only be achieved using electron-transfer reactions of **1** with organic halides (C–C bond formation) or O_2 (C–H activation). $PhCH_2Br$, PhCOCl, $NCCH_2Cl$, $BrCH_2CO_2Et$, and ICH_2CHCH_2 react in toluene or pentane at −20 °C with **1** to give orange $[(C_6Me_6)(\eta^5\text{-}C_6Me_6R)Fe^+]PF_6^-$ (**3a–e**) after metathesis with NH_4PF_6 (yields on a 1-mmol scale: $PhCH_2$ and PhCO 90%; CH_2CN 40%; $CH_2CO_2CH_3$ and CH_2CHCH_2 50%). As shown in Scheme II, the first step is an outer-sphere electron transfer from **1** to RX, characterized by the intermediate purple color and EPR spectra (g = 2.091, 2.012, 2.003 at −140 °C in acetone) of $\mathbf{1}^+$ in the reaction media before collapse to orange (**3c–e**). Thus the principle of this type of C–C bond formation lies in the coupling (within the solvent cage) of organic (R·) and organometallic (19-electron $\mathbf{1}^+$) radicals. Note that this type of activation is not suitable starting from 19-electron[7] complexes since electron transfer to RX leaves an 18-electron complex that cannot couple, thus limiting the yield of coupling products to 50%.[8]

The reaction of aqueous $H^+PF_6^-$ does not follow this course because H· atoms collapse rapidly to H_2 and $\mathbf{1}^+PF_6^-$ is formed

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