Figure 14b was not reproducible due to decomposition of Cd-Mb with high-speed MAS spinning. Apparently, the centrifugal pressure compressed the D_2O-reequilibrated protein beyond that which its structure could withstand. The original moist powder of D_2O-equilibrated Cd-Mb was transformed into an insoluble, hard, polymeric material upon spinning. We are currently trying to obtain MAS spectra of the quality needed for fitting by simulation. The static powder ^{113}Cd NMR spectrum of Cd-Mb is shown in Figure 15d. We have estimated the shielding tensor parameters from the powder spectrum and they are summarized in Table IV. Although disorder of the porphyrin in the heme pocket may result in less well resolved discontinuities of the powder pattern, it is immediately evident that the anisotropy of the chemical shift tensor has changed sign relative to Cd-PPIXDME, Cd-PPIXDME-PYR, and Cd-PPIX. The in-plane tensor elements, $\sigma_\perp \simeq 520$ ppm, are now deshielded relative to the unique element, $\sigma_\parallel = 330$ ppm, which is opposite to all previous examples of pyridyl adducts of cadmium porphyrins.[6] The isotropic chemical shift of Cd-PPIXDME-PYR, which most closely models Cd-Mb, is deshielded by only 13 ppm relative to solid Cd-Mb. In solution state, the isotropic shift of Cd-PPIXDME-PYR is shielded by 18 ppm relative to Cd-Mb. Again, if only the solution-state data were available, we would conclude, incorrectly, that Cd-PPIXDME-PYR and Cd-Mb were very similar structurally and electronically. The power of solid-state NMR is again demonstrated by exploiting the information contained in the shielding tensor parameters. In this case, solid-state ^{113}Cd NMR sensitively distinguishes pyridine from a protein histidine residue as an axial fifth ligand to Cd-PPIX, not by the isotropic chemical shift but by the sign of the anisotropy. In the case of Cd-PPIXDME, axial pyridine coordination results in shielding of the unique tensor element by 182 ppm and deshielding of the in-plane elements by ~83 ppm, i.e., the tensor elements have moved toward each other on the chemical shift scale. If the strength of the axial interaction is increased beyond that of an axial pyridine ligand, one can envision the tensor elements crossing over at some point. In Cd-Mb, the histidyl residue coordinates axially through nitrogen to the cadmium atom in such a way as to cause such a crossover to occur. The strong axial Cd-N interaction is not completely surprising, since in native myoglobin the Fe-N bond is the single covalent interaction through which the prosthetic porphyrin molecule is bound to the globin.

Conclusions

Solution- and solid-state ^{113}Cd NMR parameters have been measured in order to characterize dynamical and structural consequences of axial ligation in Cd^{2+}-coordinated protoporphyrin IX complexes, i.e., immediate precursors of Cd^{2+}-substituted hemoglobins. Solution-state results indicate that the Cd^{2+} ion undergoes fast exchange in Cd-Mb with and without axial proximal histidyl nitrogen coordination to the cadmium porphyrin. In the solid state, axial coordination of histidine to Cd-PPIX results in an anisotropy of −200 ppm, whereas axial coordination of pyridine to Cd-PPIXDME is distinguished by an anisotropy of +163 ppm. The change in the sign of the anisotropy reflects the difference in (1) the Lewis basicity of the axial ligand and (2) the Cd-axial ligand bond length. Therefore, solid-state ^{113}Cd NMR has been shown to be sensitive to the presence, identity, and bond length of the axial coordination ligands to cadmium porphyrins. In future work, cadmium porphyrins should provide a novel probe with which to examine axial dynamical and structural changes in Cd^{2+}-substituted hybrid hemoglobins. Furthermore, the expansion of solution- and solid-state ^{113}Cd NMR into investigation of other hemoproteins is promising.

Acknowledgment. We gratefully acknowledge the support from the National Institutes of Health via Grant GM26295. Further, we would like to acknowledge the helpful suggestions of Drs. Paul Marchetti and Raja Khalifah.

Registry No. Cd-PPIXDME, 14729-09-0; Cd-PPIX, 80216-25-7; PPIXDME, 5522-66-7; Cd-PPIXDME-PYR, 119818-85-8; PYR, 110-86-1; ^{113}Cd, 14336-66-4.

Model Systems for Rhodopsins: The Photolysis of Protonated Retinal Schiff Bases, Cyanine Dye, and Artificial Cyanine–Bacteriorhodopsin

Noga Friedman,[†] Mordechai Sheves,*[,†] and Michael Ottolenghi*[,‡]

Contribution from the Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel, and the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel. *Received September 6, 1988*

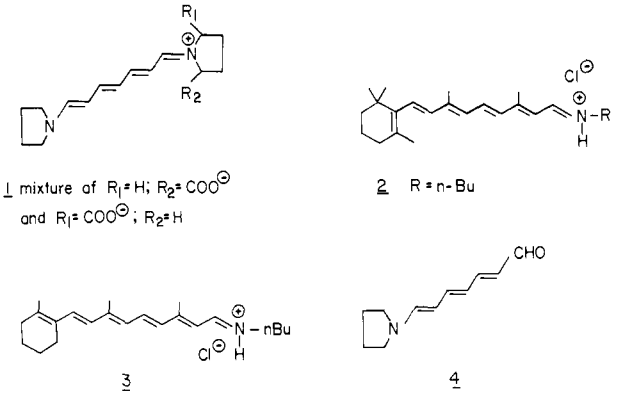
Abstract: Protonated Schiff bases of retinal ($RSBH^+$), of its (planar) linear polyene analogue 1,1-didemethylretinal ($LRSBH^+$), and of an analogous cyanine dye (Cy^{III}) are submitted to pulsed laser photolysis over a range of solvents and temperatures. Transient phenomena observed with the Cy^{III} dye are attributed to trans → cis isomerization, followed by secondary excitation which induces rotation about an additional bond. In the cases of $RSBH^+$ and $LRSBH^+$ (photostationary mixtures of cis-trans isomers), laser excitation of deaerated solutions leads to the observation of triplet states. The latter are formed via intersystem crossing (ISC) from a short-lived excited state, generated by multiple excitations of the ground state during the (same) intense laser pulse. O_2 saturation of the solutions suppresses the ISC route, giving rise to a short-lived phototransient observed at low temperatures, which is identified as a C-C conformer. The observations are discussed in light of the possibility that C-C conformers may play a role in the photocycles of visual rhodopsins and of bacteriorhodopsin. Experiments were also performed with an artificial bacteriorhodopsin pigment (bR_{cy}) carrying a cyanine chromophore analogous to Cy^{III}. A single photointermediate (bR_{cy}/I), reminiscent of the K phototransient of bR, is observed and is attributed to a cis isomer. The low activation and preexponential parameters which characterize the thermal relaxation of bR_{cy}/I are discussed in terms of cis → trans isomerization in a rhodopsin binding site. The results bear on the thermal 13-cis → all-trans relaxation in the final stages of the bR photocycle and on the inefficiency of a back (all-trans → 11-cis) reaction at the early (bathorhodopsin) stage of the visual photocycle.

Visual pigments and bacteriorhodopsin (bR), the purple-membrane pigment in the photosynthetic microorganism *Halobacterium halobium*, are both composed of a retinylpolyene chromophore bound to the parent protein (opsin) via a protonated Schiff base bond with a lysine residue. It is now well established that in both systems the photocycle is initiated by primary isom-

0002-7863/89/1511-3203$01.50/0

erization around a polyene double bond.[1] The subsequent steps initiate the transduction process (in visual pigments) or a cross-membrane proton pump (in bacteriorhodopsin). Apart from the primary isomerization, such steps involve changes in the polyene, in the opsin, and in polyene–opsin interactions, which are not fully understood. Single bond rotations in the polyene and changes in electrostatic interactions between the polyene and nonconjugated protein charges or dipoles have been suggested to account for the various spectrally distinct steps in the photocycle of a pigment such as bR.[1]

Understanding the light-induced molecular changes in the biological systems is intimately related to the photochemical behavior of the parent-protonated Schiff bases of retinals ($RSBH^+$) in solution. Considerable information is now available concerning the rates and yields of C=C cis–trans photoisomerization of $RSBH^+$ isomers.[2,3] However, single bond rotations have not yet been detected in these systems. In the present work experiments are carried out with the protonated retinal Schiff base **2** as well as with its planar (linear) analogue 1,1-didemethylretinal **3** ($LRSBH^+$) (see Scheme I). The interest in a molecule such as **3** is based on recent ^{13}C NMR studies[9a] showing that the retinal chromophore in the bR binding site adopts a planar, s-trans, ring-chain conformation, in variance with the twisted s-cis conformation which characterizes both $RSBH^+$ in solution and bovine rhodopsin.[9b] It thus appears relevant to study the planar molecule **3** which, due to the lack of the 1,1-dimethyl groups, adopts an s-trans, ring-chain conformation analogous to that of the bR chromophore. Pulsed laser excitation of both systems yield light-induced relaxation processes which are attributed to single bond isomerization reactions.



1 mixture of R_1=H; R_2=$COO^\ominus$ and R_1=$COO^\ominus$; R_2=H

2 R = n-Bu

3

4

A second aspect of the present investigation is related to the effects of polyene–protein electrostatic (and H-bonding) interactions on the spectra of the photocycle intermediates. Powerful tools in this respect, for both visual rhodopsin and bacteriorhodopsin, are artificial pigments prepared by replacing the native retinal chromophore by suitable synthetic analogues. (For recent reviews see ref 10a,b.) In the present work we have prepared an artificial bR pigment, bR_{cy}, in which the all-trans retinyl moiety of the native pigment has been replaced by the cyanine dye system of molecule **4**. (See ref 10c,d for previous spectroscopic work on artificial bR, based on a cyanine dye chromophore.) The chromophores of bR_{cy} and its model compound in solution Cy^{III} (**1**) are closely related to a variety of aromatic cyanine dyes which arose with considerable interest due to their application as laser media and to their role as model systems for cis–trans photo-

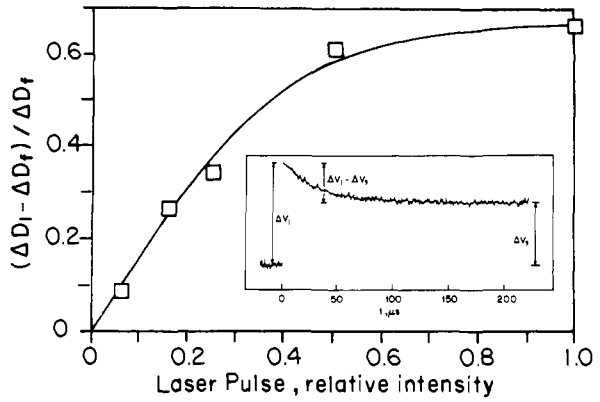
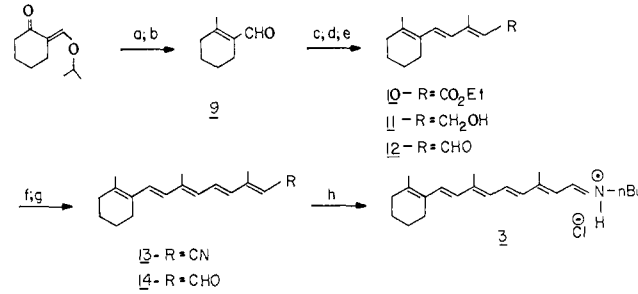


Figure 1. Characteristic trace (insert) due to transient absorbance changes of Cy^{III} in CH_2Cl_2 at 20 °C following 530-nm pulsed laser excitation (detection wavelength: 590 nm). ΔV_i and ΔV_f represent the "initial" and the "final" (following the primary decay) voltage changes, from which the corresponding absorbance changes ΔD_i and ΔD_f were calculated. The curve shows the ratio between the relative yield of the short-lived transient Cy^{III}/S (measured by $\Delta D_i - \Delta D_f$) and the long-lived transient Cy^{III}/L (measured by ΔD_f) as a function of the laser pulse intensity (measured as fractions of ~1 mJ/cm, set by using appropriate neutral density filters).

Scheme I[a]



[a] (a) MeLi, ether, 0 °C, 1 h. (b) HCl, EtOH, 25 °C, 2 h. (c) $(EtO)_2POCH_2C(CH_3)=CCO_2Et$, NaH, THF, 25 °C, 1 h. (d) DIBAL, THF, −78 °C, 2 h. (e) MnO_2, CH_2Cl_2, 25 °C, 18 h. (f) $(EtO)_2POCH_2C(CH_3)=CCN$, THF, 25 °C, 15 min. (g) DIBAL, hexane, −78 °C, 2 h; wet silica for 2 h. (h) $BuNH_2$, EtOH, 25 °C, 30 min, followed by evaporation of solvent and protonation with HCl.

isomerization studies.[4-8] Our particular choice of the nonaromatic bR_{cy} and Cy^{III} was based on their characteristic of exhibiting an absorption spectrum which is essentially insensitive to external (nonconjugated) charges.[11] Thus, bR_{cy} can be used to probe photoisomerization processes in the binding site which are not masked by changes in polyene–protein electrostatic interactions. Another interesting aspect of bR_{cy} is the intrinsic pK_a value of its cyanine chromophore which is expected to be analogous to that of Cy^{III} and thus substantially higher than those of $RSBH^+$ and bR, respectively.[12] This may affect the deprotonation step which

(1) For reviews of rhodopsin photochemistry see: (a) Ottolenghi, M. In *Advances in Photochemistry*; Pitts, J., Ed.; Wiley: New York, 1980; Vol. 12, pp 97–200. (b) Birge, R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 315. (c) *Methods in Enzymology, Biomembranes P.I. Visual Pigments and Purple Membranes, I, II*; Packer, L., Ed.; Academic Press: 1982. (d) Becker, R. S. *Photochem. Photobiol.* **1988**, *48*, 369.

(2) (a) Becker, R. S.; Freedman, K. *J. Am. Chem. Soc.* **1985**, *107*, 1477. (b) Becker, R. S.; Freedman, K.; Hutchinson, J. A.; Noe, J. L. *Ibid.* **1985**, *107*, 3942. (c) Freedman, K. A.; Becker, R. S. *Ibid.* **1986**, *108*, 1245 and references therein.

(3) Childs, R. F.; Shaw, G. S. *J. Am. Chem. Soc.* **1988**, *110*, 3013.

(4) Rulliere, C. *Chem. Phys. Lett.* **1976**, *43*, 303.

(5) Kuzmin, V. A.; Darmanyan, A. P. *Chem. Phys. Lett.* **1978**, *54*, 159.

(6) Velsko, P. S.; Graham, R. F. *Chem. Phys.* **1982**, *65*, 59.

(7) Dietz, F.; Rentsch, S. K. *Chem. Phys.* **1985**, *96*, 145.

(8) (a) Akesson, E.; Sundstrom, V.; Gillbro, T. *Chem. Phys. Lett.* **1985**, *121*, 513. (b) Akesson, E.; Bergstrom, H.; Sundstrom, V.; Gillbro, T. *Ibid.* **1986**, *126*, 385.

(9) (a) Harbison, G. S.; Smith, S. O.; Pardoen, J. A.; Courtin, J. M.; Lugtenburg, J.; Herzfeld, J.; Mathies, R.; Griffin, R. *Biochemistry* **1985**, *24*, 6955. (b) Smith, S. O.; Palings, I.; Copie, V.; Raleigh, O.; Courtin, J.; Pardoen, J. A.; Lugtenburg, J.; Mathies, R. A.; Griffin, R. G. *Biochemistry* **1987**, *26*, 1606.

(10) (a) Derguini, F.; Nakanishi, K. *Photochem. Photobiophys.* **1986**, *13*, 259. (b) Sheves, M.; Ottolenghi, M. In *Chemistry and Biology of Synthetic Retenoids*; CRC Press; in press. (c) Derguini, F.; Caldwell, C. G.; Motto, M. G.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 646. (d) Kakitani, T.; Kakitani, H.; Honig, B. *Ibid.* **1983**, *105*, 648.

(11) Sheves, M.; Friedman, N. *Angew. Chem., Int. Ed., Engl.* **1986**, *25*, 284.

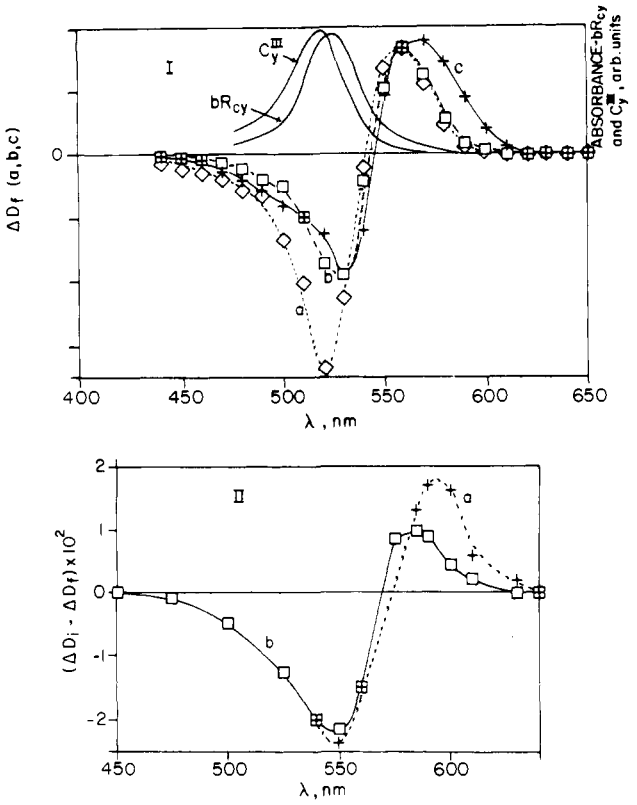


Figure 2. I. Absorption spectra at 20 °C of bR_{cy} and Cy^{III} (**1**) (in ethanol) and their 530-nm pulsed laser photolysis difference spectra: a, b, Cy^{III}/L in PrOH and CH_2Cl_2, respectively; c, bR_{cy}/I. (Scale for ΔD_f is omitted since curves b and c were arbitrarily normalized to the maximum positive ΔD_f value of curve a.) II. Cy^{III}/S: (a) generated by 530-nm laser excitation of Cy^{III} in CH_2Cl_2 at 20 °C (see Figure 1) and (b) generated by accumulation of Cy^{III} in PrOH at −70 °C by continuous illumination, followed by 530-nm laser excitation.

is associated with the M intermediate in the photocycle of bR. In the absence of deprotonation, one may expect a relatively simpler photocycle which will more directly allow the investigation of light-induced isomerizations.

Results

(a) Cyanine Dye Cy^{III} and the Analogous bR Pigment, bR_{cy}. Pulsed laser photolysis of aerated solutions of the cyanine molecule Cy^{III} (**1**) yields transient absorbance changes as shown by the characteristic trace in Figure 1, insert. In both methylene chloride and methanol an increase in absorbance is observed in the red, exhibiting a two-component decay, attributed to a short-lived and to a long-lived photolysis transient, respectively. The corresponding absorbance changes, defined as $(\Delta D_i - \Delta D_f)$ and ΔD_f (where ΔD_i is the initial absorbance change and ΔD_f is that observed after completion of the initial decay), are plotted in Figure 2, II and I, respectively. It is evident that the difference spectrum corresponding to the short-lived cyanine intermediate (Cy^{III}/S) is red-shifted relative to the long-lived component, Cy^{III}/L, by approximately 30 nm. The above photolysis patterns are insensitive to saturating the solution with either O_2 or N_2.

Experiments were also carried out monitoring the above absorbance changes as function of the laser pulse intensity. The results, shown in Figure 1, indicate that the relative amount of the fast component depends markedly on light intensity, suggesting a mechanism in which Cy^{III}/S is generated by (secondary) excitation of Cy^{III}/L. This was actually confirmed by steady-state experiments carried out at low temperatures (e.g., below −70 °C) when Cy^{III}/L becomes thermally stable (see below). As shown in Figure 2IIb, under such conditions Cy^{III}/S may be generated by accumulating Cy^{III}/L by steady-state illumination, followed by laser excitation.

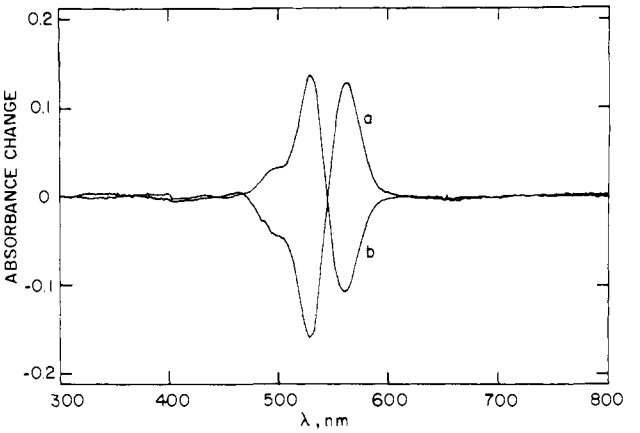


Figure 3. Difference spectra obtained by (~20 s) steady-state illumination of Cy^{III} (**1**) at −90 °C: (a) difference spectrum recorded following illumination using a 490-nm interference filter and (b) excitation of (a) using a λ >590 nm cut-off filter.

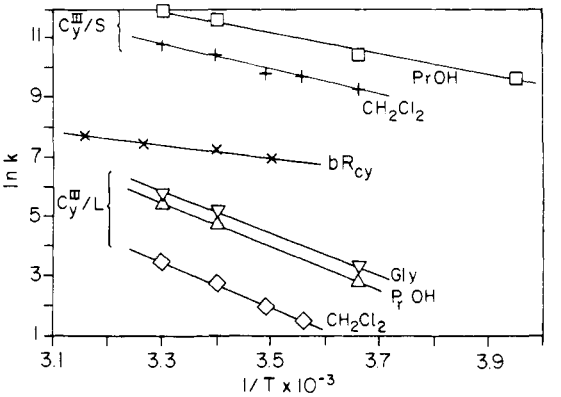


Figure 4. Arrhenius plots for the decay rate constant (k) of bR_{cy} and for Cy^{III}/S and Cy^{III}/L in a variety of solvents [*n*-propanol (PrOH), glycerol (Gly), methylene chloride (CH_2Cl_2)].

Table I. Rate Constants, k(20 °C), and Corresponding E_a and A Parameters for the Decay of Phototransients in the Pulsed Photolysis of Cy^{III} and bR_{cy}[b]

photo-transient	solvent and viscosity (cp, at 20 °C)	k, s^{-1}	E_a, Kcal/M	A
Cy^{III}/L	CH_2Cl_2 (0.5)	14.7	15.1 ± 0.5	1.9×10^{12}
	PrOH (2.2)	1.1×10^2	14.8 ± 0.5	9.7×10^{12}
	Gly (1500)	1.6×10^2	13.4 ± 0.5	1.3×10^{12}
Cy^{III}/S	CH_2Cl_2 (0.5)	3.3×10^4	8.5 ± 0.5	6×10^{10}
	PrOH (2.2)	1.15×10^5	6.2 ± 0.5	3×10^9
	Gly (1500)[a]			
bR_{cy}/I		1.4×10^3	5.2 ± 0.4	9×10^6

[a] Transient not observed. [b] Solvents: methylene chloride (CH_2Cl_2), propanol (PrOH), glycerol (Gly).

Figure 3 shows that Cy^{III}/L is photoreversible. Thus, steady-state illumination at −90 °C with short wavelength light induces the $Cy^{III} \rightarrow Cy^{III}/L$ transition. The effect is reversed by exposure to longer wavelength light.

Pulsed photolysis of the artificial cyanine bacteriorhodopsin pigment bR_{cy} derived from **4**[11] yielded a single transient decay associated with a red-shifted intermediate bR_{cy}/I. The corresponding light-induced difference spectrum is given in Figure 2Ic.

The decay rates of Cy^{III}/S and Cy^{III}/L in a variety of solvents as well as those of bR_{cy}/I were measured as a function of temperature. The corresponding Arrhenius plots and rate parameters are shown in Figure 4 and Table I. As shown in Table I the decay of Cy^{III}/L shows little dependence on viscosity. Cy^{III}/S was undetectable in high viscosity solvents such as propylene glycol or glycerol.

(12) (a) Schaffer, A. M.; Yamaska, T.; Becker, R. S. *Photochem. Photobiol.* **1975**, *21*, 279. (b) Druckmann, S.; Ottolenghi, M.; Pande, J.; Callender, R. H. *Biochemistry* **1982**, *21*, 4953.

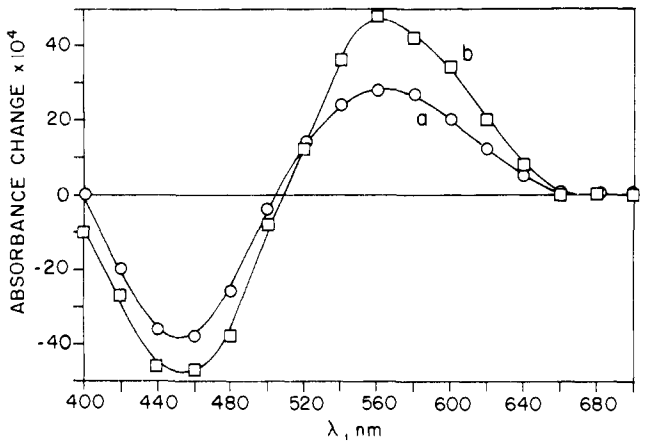


Figure 5. Transient absorbance changes following 530-nm pulsed laser excitation of deaerated solutions of (a) $RSBH^+$ (0 °C) and (b) $LRSBH^+$ in PrOH (−70 °C).

(b) Retinal (2) and 1,1-Didemethylretinal (3) Protonated Schiff Bases and Related Compounds. $LRSBH^+$ (1,1-didemethylretinal) was synthesized[13] from 1-formyl-2-methylcyclohexene (**9**) by applying the Emmons–Horner reaction with the sodium salts of triethyl 3-methyl-4-phosphonocrotonate and diethyl 3-methyl-4-phosphonocrotononitrile followed by reduction with diisobutylaluminum hydride (Scheme I). The consequences of pulsed laser excitation of the *n*-butylamine (*n*-Bu) protonated Schiff bases of **2** and **3** were investigated in deaerated solutions of the respective all-trans (or 11-cis) isomers which were previously pre-exposed to several laser pulses so as to achieve the same (undefined) photostationary mixture of trans and cis isomers. Under such conditions no net changes in absorbance, due to cis-trans isomerization[2] are induced by the laser pulse. Figure 5 shows transient difference spectra for both $RSBH^+$ and $LRSBH^+$ in PrOH. Similar observations were made in a variety of deaerated solvents (e.g., methylpentane, methylene chloride), temperatures, and excitation wavelengths (e.g., 355, 420, 530 nm). In all cases a red-shifted intermediate is observed associated with an ~570-nm maximum (~500 nm isosbestic point) in the difference spectrum. The phototransient, exhibiting a half-life of $\sim 3.5 \times 10^{-5}$ s (for $LRSBH^+$ in PrOH at room temperature), was undetectable (within the ~50 ns time resolution of the laser system) in oxygen-saturated solutions.

In view of the resemblance of the difference spectra of Figure 5 to those characterizing the triplet state of $RSBH^+$ as generated by Fisher and Weiss via energy transfer in methylclohexane,[14] we performed laser photolysis experiments in mixed anthracene (donor) $RSBH^+$ and $LRSBH^+$ (acceptors) systems. The results, shown in Figure 6 for $LRSBH^+$, refer to systems in which most of the exciting laser beam (>90%) is absorbed by anthracene. It is evident that in methylpentane, the initial absorbance change characteristic of the 435-nm anthracene triplet state gradually gives rise to difference spectra due to the triplet state of the acceptor.[14] Similar results were obtained for $RSBH^+$. As shown in Figure 6II the efficiency of triplet energy transfer decreases with solvent polarity. No transfer could be detected in ethanol. In view of the close similarity between these transient spectra and decay kinetics and those observed in the direct-excitation experiments of Figure 5, we conclude that the latter are due to the generation of the triplet states of either $RSBH^+$ or $LRSBH^+$.

Looking for transient phenomena (other than triplet-state formation) which may be analogous to those observed with the cyanine dye, the experiments with photostationary isomer mixtures of $RSBH^+$ and $LRSBH^+$ were extended to low temperatures in a variety of oxygen-saturated solvents. In the presence of oxygen

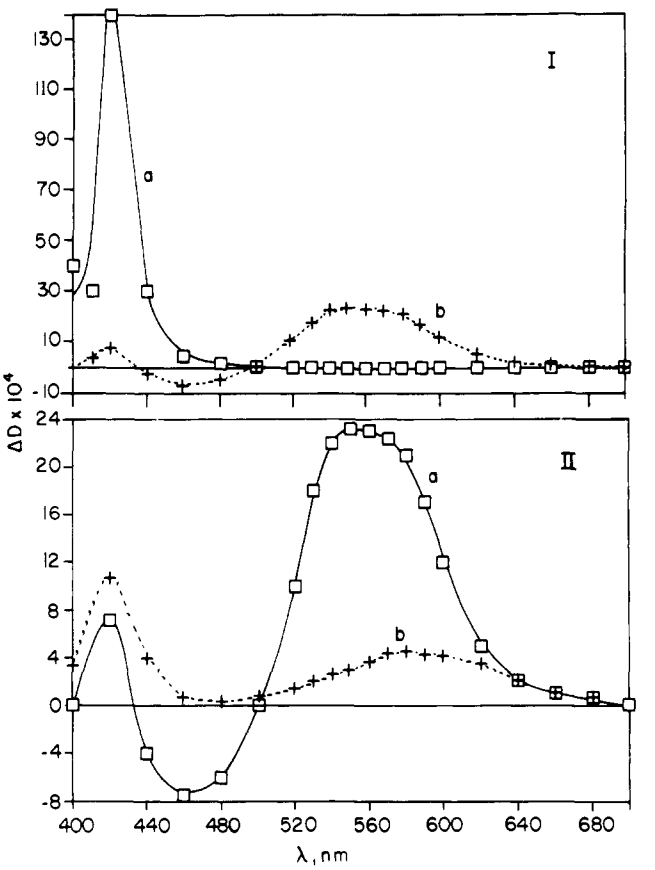


Figure 6. Transient absorbance changes showing energy transfer from anthracene triplet to $LRSBH^+$ in deaerated solutions at 20 °C: (I) in MePe, 1 μs (a) and 17 μs (b) after 355-nm excitation; (II) 17 μs after 355-nm excitation in (a) MePe (same as Ib) and (b) in CH_2Cl_2.

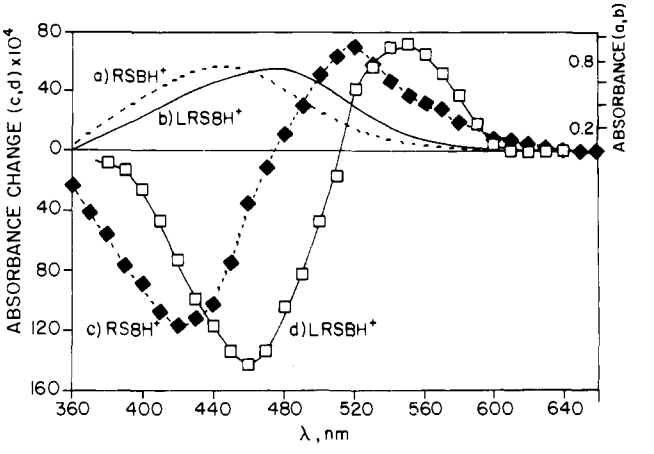


Figure 7. Absorbance of $RSBH^+$ (a) and $LRSBH^+$ (b) (in PrOH at 20 °C) and the corresponding transient absorbance changes (c and d) measured 2 μs after 420- and 530-nm excitation (respectively) of O_2-saturated solutions at −70 °C.

the characteristic absorbance change due to the triplet state is replaced by a new, shorter-lived transient whose lifetime is independent of the oxygen concentration. Experiments carried out in air-saturated solutions indicate that the O_2 effect is not limited to quenching of the $RSBH^+$ and $LRSBH^+$ triplet states. It is primarily associated with a decrease in the triplet yield. Figure 7 shows the corresponding difference spectra, for both $RSBH^+$ and $LRSBH^+$, with maxima at 515 and 545 nm, respectively. They reflect bleaching of the corresponding polyene band and generation of the new (red-shifted) intermediates which we denote as $RSBH^+/I$ and $LRSBH^+/I$, respectively. Figure 8 shows an Arrhenius plot for the (first-order) decay rate constant of $LRSBH^+/I$, carried out in a set of alcoholic mixtures with the

(13) 1,1-Didemethylretinal was synthesized by a method similar to that described by the following: Courtin, J.; Verhagen, L.; Biesheuvel, P.; Lugtenburg, J.; Van der Bend, R. L.; Van Dam, K. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 112.

(14) Fisher, M.; Weiss, K. *Photochem. Photobiol.* **1974**, *20*, 423.

Table II. Rate Parameters for the Decay of $RSBH^{+\cdot}/I$ or $LRSBH^+/I$ and Related Systems

compound	base	counterion	solvent	viscosity (−70 °C) cp	λ_{max}, nm	k, s^{-1} (−70 °C)	E_a, kcal/M	A
$RSBH^+$ (3)	*n*-$BuNH_2$	Cl^-	PrOH	70	446	1.4×10^5	3.7 ± 0.2	9.8×10^8
$RSBH^+$ (2)	*n*-$BuNH_2$	Cl^-	CH_2Cl_2		465	1.7×10^4		
$RPPC^+$ (5)		ClO_4^-	CH_2Cl_2		490	1.8×10^4		
$RPRO^+$ (6)		COO^-	CH_2Cl_2		475	1.6×10^4		
$LRSBH^+$ (3)	*n*-$BuNH_2$	Cl^-	MeOH	4.3	474	1.7×10^5	6.4 ± 0.1	1.2×10^{12}
$LRSBH^+$ (3)	*n*-$BuNH_2$	Cl^-	EtOH	13	474	1.4×10^5	7.2 ± 0.6	7.2×10^{12}
$LRSBH^+$ (3)	*n*-$BuNH_2$	Cl^-	PrOH	70	474	1.0×10^5	6 ± 0.2	2.1×10^{11}
$LRSBH^+$ (7)	*t*-$BuNH_2$	Cl^-	PrOH	70	470	1.1×10^5		
$LRSBH^+$ (8)	Adam·NH_2	Cl^-	PrOH	70	470	1.0×10^5		

[a] E_a and A are the apparent Arrhenius activation and preexponential factors calculated by assuming $k = A \exp(-E_a/RT)$. [b] $E_0 = 5.5 \pm 0.4$ Kcal/M and $A' \simeq 1.8 \times 10^{11}$ for $LRSBH^+$ (3) have been estimated by assuming $k = (A'/\eta^a) \exp(-E_0/RT)$ as described in the text.

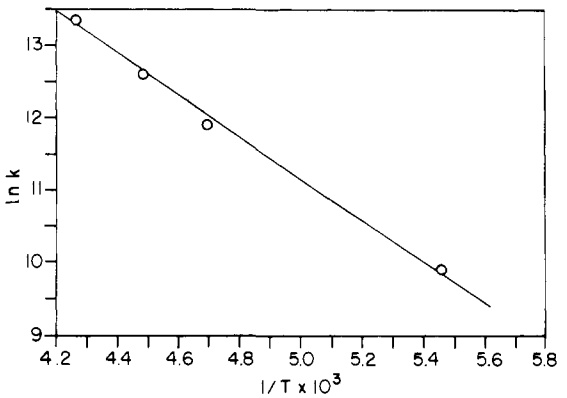
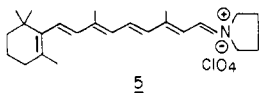


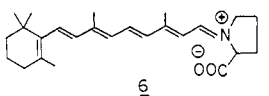
Figure 8. Arrhenius plot of the temperature dependence of the decay rate constant (k) of $LRSBH^+/I$ in isoviscous solutions (propanol, −60 °C; butanol, −50 °C; ethanol, −90 °C): log $\eta = 1.5 \pm 0.1$.

same viscosity, η. Figure 9 gives a set of ln k vs ln η plots at two (constant) temperatures.

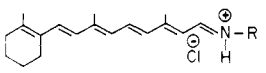
The high sensitivity of $RSBH^+$ spectra to the interactions between the protonated Schiff base nitrogen and its counterion is well established.[15,16] Thus, for probing the nature of the $RSBH^+/I$ and $LRSBH^+/I$ we carried out photolysis experiments with the analogous systems, pyrrolidine perchlorate **5** and the proline derivative **6**. The latter carries a fixed counterion which markedly



5



6



7 R = t-Bu

8 R = 1-Adamantyl

reduces the degree of freedom along the nitrogen–counterion coordinate.

For similar reasons we have also investigated the *t*-Bu amine and adamantyl (Ad) Schiff bases of **2** and **3** (denoted as **7** and **8**, respectively), which are both characterized by increased steric restrictions in the Schiff base environment, as compared with the corresponding *n*-Bu amine Schiff bases. For all of the systems **5–8** the transient photolysis patterns (yields and kinetics) associated with generation of the triplet state and of the $RSBH^+/I$ and $LRSBH^+/I$ phototransients, were very similar to those observed with the corresponding *n*-Bu amine systems of $RSBH^+$ and $LRSBH^+$. The major kinetic parameters are given in Table II.

Discussion

(a) The Cyanine Dye (Cy^{III}). The most plausible identification of Cy^{III}/L, the long-lived photointermediate of all-trans Cy^{III}, is

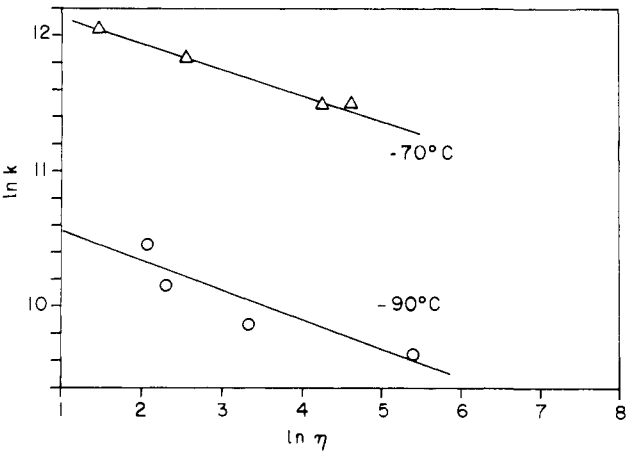


Figure 9. Viscosity dependence of the decay rate constant (k) of $LRSBH^+$ in MeOH, EtOH, PrOH, BuOH, at −70 °C and −90 °C.

a cis isomer. This is in keeping with the photoreversibility of Cy^{III}/L which is characteristic of cis↔trans photoprocesses. Also relevant are the analogies with the photoisomerization of parent carbocyanine and polymethine dyes.[4-8] These refer both to the magnitude of the activation energy of the thermal back reaction (of the order of 14 Kcal in the present system as compared to 10–14 Kcal for the above dyes), to the "normal" pre-exponential factors ($A = 10^{11}$–10^{13} s^{-1}), and to the insensitivity of both parameters to the dielectric constant. We note that as in the case of the above cyanine dyes, we cannot at present identify the specific bond associated with the Cy^{III}/L photoproduct. Thus, calculated bond orders for the ground state of cyanine dye similar to **1** show a uniform value of 0.6–0.7 for all C,C bonds (~0.5 for the C,N bonds).[17] This is qualitatively in keeping with our observed activation energy, but qualifies any C,C or C,N bond for the purposed isomerization in Cy^{III}/L.

As to the nature of Cy^{III}/S, we suggest identifying it as a dicis conformer. A similar assignment has been made to account for fast decaying (lower activation energy) phototransients of several polymethine dyes.[5] Accordingly, we propose the scheme

$$Cy^{III}(\text{trans}) \underset{h\nu_2 \text{ or } k_1}{\overset{h\nu_1}{\rightleftharpoons}} Cy^{III}/L\ (\text{cis}) \underset{k_2}{\overset{h\nu_2}{\rightleftharpoons}} Cy^{III}/S\ (\text{dicis})$$

(b) The Artificial bR-Cyanine Pigment (bR_{cy}). It is well established that the photocycle of bacteriorhodopsin is based on a primary trans → cis isomerization.[1,18-20] It is thus tempting to identify the photointermediate bR_{cy}/I, observed for the artificial bR pigment derived from **4**, as analogous to one of the suggested photoisomers of the parent cyanine dye Cy^{III}. The close similarity between the difference spectra of bR_{cy}/I and Cy^{III}/L (see Figure

(15) Blatz, P. F.; Mohler, J. H. *Biochemistry* **1972**, *11*, 3240.
(16) Sheves, M.; Kohne, B.; Mazur, Y. *J. Chem. Soc., Chem. Commun.* **1983**, 1232.
(17) Klessinger, K. *Theor. Chim. Acta* **1966**, *5*, 251.
(18) Braiman, M.; Mathies, R. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *79*, 403.
(19) Fang, J. O.; Carriker, J.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 5162.
(20) Albeck, A.; Friedman, N.; Sheves, M.; Ottolenghi, M. *J. Am. Chem. Soc.* **1986**, *108*, 4614.

2I) and the monophotonic route to bR_{cy}/I would suggest that the latter is associated with a cis conformation analogous to Cy^{III}/L. We note, however, that using the spectra of the two systems as a criterion is plausible only if specific spectroscopic effects of the protein environment, primarily of nonconjugated charges and of H-bonds, may be neglected. The latter are well-known to markedly affect the spectra of bacteriorhodopsin and of visual pigments by interacting with their respective retinyl chromophores.[21-25] However, in view of the low spectral sensitivity of cyanine dyes such as **1** to nonconjugated charges and H-bonds,[11] much smaller protein effects are expected in the case of bR_{cy} and its photoproduct, thus validating the above spectral criterion. It should be pointed out, however, that the second alternative, namely that bR_{cy}/I involves a dicis conformation in analogy to Cy^{III}/S, is unlikely (mainly because of the large change in geometry) but cannot be definitely ruled out. Nevertheless, for the sake of the subsequent discussion we adopt the trans → monocis isomerization assumption.

The assumption that bR_{cy}/I involves a trans → cis isomerization makes it analogous to the cis, $C_{13}{=}C_{14}$, conformation of the photoisomerized retinyl chromophore in the photocycle of all-trans (light-adapted) bacteriorhodopsin,1 more specifically, to the red shifted K photointermediate of bR. Consequently, questions obviously arise as to why only a single step is observed in the photocycle of bR_{cy} and, especially, as to the mechanism by which the protein catalyzes the thermal back-reaction ($bR_{cy}/I \rightarrow bR_{cy}$) in respect to that ($Cy^{III}/L \rightarrow Cy^{III}$) of the analogous free pigment in solution. In relation to the first question we note that a multistage photocycle analogous to that of bR may actually be taking place in the case of bR_{cy} without being detectable by optical spectroscopy, due to the insensitivity of the cyanine polyene system to (environmental) protein electrostatic effects. FTIR techniques, capable of monitoring changes in the protein, may be applied, as in the case of bR,[26] to detect stages in the photocycle occurring during the lifetime of bR_{cy}/I. The lack of a Schiff-base deprotonation step, analogous to the M intermediate in the bR photocycle, is feasible in view of the relatively high pK_a values of symmetrical cyanine dye such as Cy^{III} ($pK_a = 12.5 \pm 0.2$ vs $pK_a = 7.4 \pm 0.2$ for the retinal Schiff base[12] in 50% CH_3OH–H_2O).

In relation to the second question, we should consider the relatively fast, low activation barrier, decay of bR_{cy}/I as compared to that of Cy^{III}/L. The effect is due both to a lower activation energy as well as to a substantially lower preexponential factor (see Table I). Obviously, it is possible that the rate-determinating step in the decay of bR_{cy}/I is not the intrinsic C,C; cis → trans isomerization but rather a protein conformational change which catalytically induces the subsequent, much faster, C,C isomerization. In such a case a substantial protein-induced decrease in the isomerization barrier E_a must be implied, but no quantitative estimate of E_a will be available. Moreover, the apparent A value will not reflect the actual isomerization preexponential factor. It should thus be emphasized that the following discussion concerning the protein effect on E_a qualitatively applies, independently of whether or not the C,C isomerization is rate determining. On the other hand, the discussion concerning the low value of A applies only if the rate-determining step in the decay of bR_{cy}/I is the cis → trans isomerization of the polyene.

Addressing the activation barrier (E_a) first, we note that, as in the case of rhodopsins,[27-29] a substantial fraction of the photon

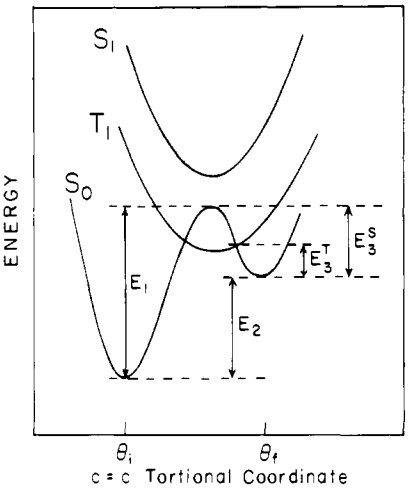


Figure 10. Schematic potential energy surfaces (see ref 1a) describing photoisomerization in rhodopsins. The pigment in its initial configuration θ_i (11-cis in visual rhodopsin; all-trans in light-adapted bR) is excited from the lowest singlet state, S_0, to the S_1 excited state. Following motion along the corresponding tortional coordinate ($C_{11}{=}C_{12}$ and $C_{13}{=}C_{14}$, respectively) and crossing to S_0 the final configuration, θ_f, is obtained, corresponding to all-trans and 13-cis for visual rhodopsin and bR, respectively. θ_i is all-trans and θ_f is an undefined cis in the case of bR_{cy}. E_1 is the ground-state barrier for thermal isomerization, E_2 is the energy stored in bathorhodopsin (~32 Kcal/mol) or K (~14 Kcal/mol). E_3^T and E_3^s are the barriers for the thermal back-reaction via triplet and singlet mechanisms, respectively. E_1 in visual rhodopsins has been estimated (see Discussion by the following: Honig, B.; Ebrey, T.; Callender, R. H.; Dinur, U.; Ottolenghi, M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2503) as E_1 = 30–36 Kcal/mol. Accordingly, $E_3^s = E_1 - E_2$ is of the order of several Kcal/mol.

energy (15 Kcal/mol for the K photointermediate of bR[29]) may be stored in the bR_{cy}/I photoproduct. This energy will destabilize the latter (cis) conformer, decreasing the relaxation barrier to the (trans) Cy^{III} configuration (see Figure 10). Alternatively, the relaxation may be catalyzed by nonconjugated protein charges in the vicinity of the polyene chain.[30,31] Discrimination between the two alternatives is now under investigation in our laboratory by studying the effects of nonconjugated charges on the thermal relaxation of, e.g., Cy^{III}/L.

More intriguing is the value of the A parameter characterizing the decay of bR_{cy}/I, which is lower by a factor of 10^3–10^6 as compared with the thermal relaxations of either Cy^{III}/L or Cy^{III}/S as well as with the isomerization of a variety of cyanine dyes for both excited (trans → cis) or ground-state (cis → trans) surfaces.[5-8] It is also relevant to note that a "normal" preexponential factor, $A \simeq 10^{13}$ s^{-1} (E_a = 23 Kcal/mol), is observed for the thermal relaxation of light-adapted bR into dark-adapted bR, a process associated with a $C_{13}{=}C_{14}$ trans → cis isomerization of the retinylpolyene chromophore.[32] It is therefore evident that if the isomerization of the polyene is rate-determining in the decay of bR_{cy}/I, one is facing an anomality in A which is thus characteristic of the cyanine photointermediate in the bR binding site.

At present we envisage two alternative mechanisms to account for the low A factor. The first is based on sequential singlet (S_0) → triplet (T_1) → singlet (S_0) crossings (Figure 10), in which the probability of singlet ↔ triplet transfers must be included in the transmission coefficient of the reaction (in terms of absolute reaction rate theory). In fact, A factors of the order of 10^5–10^6 s^{-1} have been theoretically estimated to account for the unusually low preexponential parameters observed in early investigations of several thermal (uncatalyzed) cis–trans isomerizations.[33a]

(21) Kropf, A.; Hubbard, R. *Ann. N.Y. Acad. Sci.* **1958**, *74*, 266.
(22) Honig, B.; Dinur, U.; Nakanishi, K.; Balogh-Nair, V.; Gavinowicz, M. A.; Arnaboldi, M.; Moto, M. *J. Am. Chem. Soc.* **1979**, *101*, 7084.
(23) Nakanishi, K.; Balogh-Nair, V.; Arnaboldi, M.; Tsujimoto, K.; Honig, B. *J. Am. Chem. Soc.* **1980**, *102*, 7945.
(24) Spudich, J. L.; McCain, D. A.; Nakanishi, K.; Okabe, M.; Shiniger, N.; Rodman, H.; Honig, B.; Bogomolni, R. A. *Biophys. J.* **1986**, *49*, 479.
(25) Lugtenburg, J.; Muradin-Szweykowska, M.; Heeremans, C.; Pardoen, J. A.; Smith, S. O.; Mathies, R. A. *J. Am. Chem. Soc.* **1986**, *108*, 3104.
(26) See, e.g.: (a) Bagley, K.; Dollinger, G.; Einsenstein, L.; Singh, A. N.; Zimanyi, L. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 4972. (b) Siebert, F.; Maentele, W.; Krentz, W. *FEBS Lett.* **1982**, *82*, 141. (c) Rothschild, K.; Cantore, W. A.; Marrero, H. *Science* **1983**, *219*, 1333.
(27) Cooper, A. *Nature (London)* **1979**, *282*, 531.
(28) Boucher, F.; Leblanc, R. M. *Photochem. Photobiol.* **1985**, *41*, 459.
(29) Birge, R.; Cooper, T. *Biophys. J.* **1983**, *42*, 61.
(30) (a) Schulten, P.; Tavan, P. *Nature (London)* **1978**, *272*, 85. (b) Warshel, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 2558.
(31) Sheves, M.; Baasov, T. *J. Am. Chem. Soc.* **1984**, *106*, 6840.
(32) See discussion by the following: Warshel, A.; Ottolenghi, M. *Photochem. Photobiol.* **1979**, *30*, 291.

Although later data questioned the involvement of triplet states in the above (olefin) systems,[33b] recent work appears to reconfirm the triplet crossing mechanism.[33c,d] Such a mechanism is especially attractive in a protein complex such as bR_{cy}. Thus, as shown in Figure 10, the triplet surface may become thermally accessible in bR_{cy}/I due to a low E_s^T value caused by destabilization of the ground state via energy storage (E_2) in the θ_f configuration of bR_{cy}/I.

The alternative mechanism, which does not require surface crossing, attributes the low A value to a low (or negative) activation entropy. Namely, in the activated complex the polyene and its protein environment must assume a tight key-lock configuration which is associated with a decrease in entropy. The question obviously arises as to why normal A factors are observed for the thermal light to dark adaptation of bacteriorhodopsin, associated with an all-trans → 13-cis relaxation,[32] and in the excited-state isomerizations of bR and visual pigments which are both known to occur over 10^{-12}–10^{-13} s time scales.[1] (The same most probably applies to the photoisomerization of bR_{cy}.) A feasible explanation is to assume that the native retinal chromophore fits the protein binding site in a way which allows cis–trans barrier crossing in the activated complex without requiring rearrangement of the protein pocket and, consequently, a decrease in entropy. This situation may not be applicable to the artificial cyanine transient pigment bR_{cy}/I. Alternatively, a protein pocket conformation in bR_{cy}/I, which is unsuitable for isomerization, may be a result of an altered protein geometry induced by photoisomerization of the chromophore in the $bR_{cy} \rightarrow bR_{cy}/I$ step. In fact, protein conformational changes, following photoisomerization of the retinal moiety, have been suggested to account for the formation of bathorhodopsin and K from their respective red-shifted intermediates.[34] A similar alteration in protein conformation is thus assumed to characterize the bR_{cy}/I intermediate in variance with the relaxed ground states, or vertically excited states, of bR, bR_{cy}, and visual pigments. We note in this respect that it is not unlikely that an analogously low A factor may have to be invoked to account for the relative stability of bathorhodopsin, the primary (all-trans) photointermediate of (11-cis) visual rhodopsin and for the K photointermediate of bR. In the visual system the thermal trans → cis back-reaction (k_b) does not compete with the forward, bathorhodopsin → lumirhodopsin step, therefore, implying that $k_b < 10^6$ s^{-1}. No exact estimates are available for the activation parameter, E_a, associated with k_b. However, indirect evidence (see legend to Figure 10) suggests that $E_a = E_3^s = E_1 - E_2$ is of the order of only several Kcal/mol. In such a case an unusually low A factor may have to be invoked, on the basis of arguments analogous to those suggested for the $bR_{cy}/I \rightarrow bR_{cy}$ relaxation. The same applies to the K → bR back-reaction which, being unable to compete with the forward K → L process, is slower than $\sim 10^{-5}$ s. Since in this case we have no estimates of E_3, it is difficult to argue in favor of a low A factor as the cause of the inefficiency of the back-reaction.

(c) RSBH$^+$ and LRSBH$^+$. Triplet State Generation. For protonated Schiff bases in solution cis ↔ trans isomerization about double bonds has been the only detectable photoprocess.[2] Intersystem crossing (ISC) to the triplet state has not been observed by either nanosecond or picosecond spectroscopy in a variety of solvents and excitation wavelengths.[2,14] In methylcyclohexane an upper limit of 0.001 has been set for the ISC yield by Fisher and Weiss.[14]

We account for our observation of triplet states following direct excitation RSBH$^+$ and LRSBH$^+$, in terms of multiple excitations, induced by the relatively high laser power (~ 100 mJ/cm^2) used in our experiments, combined with a relatively high signal-to-noise ratio (> 6 for $\Delta D > 10^{-3}$) in our transient absorption measurements, due to the signal averaging procedure. Accordingly, triplet states are formed according to the scheme

$$RSBH^+ \underset{k_f+k_d}{\overset{h\nu}{\rightleftharpoons}} {}^1RSBH^{+*} \xrightarrow{k_{ISC}} {}^3RSBH^{+*}$$

In light of the ultrafast decay of the fluorescent singlet state $^1RSBH^{+*}$ ($k_d + k_f > 10^{11}$ s^{-1}, where k_f and k_d denote the radiative and nonradiative decays, respectively[2a]) in respect to the $\sim 10^{-8}$-sec laser pulse, multiple excitation of RSBH$^+$ is feasible provided that the light intensity (h, in photons/cm^2) is sufficiently high so that each molecule in the exposed solution can be excited numerous times. The validity of this condition in our experimental setup can be tested in light of the Beer–Lambert law: $-dh = h\sigma n dl$, where dl is the (differential) optical path, n is the absorbing solute (RSBH$^+$) concentration in molecules/cm^3, and σ is the absorption crossection in cm^2 (since $e^{-\sigma nl} = 10^{-\epsilon cl}$, where c is the concentration in mol/L, we have $\sigma = 3.82 \times 10^{-21}\,\epsilon$). The probability that a molecule will absorb a photon is given by the ratio between the density of photons (dh/dl) and the density of absorbing molecules, n, i.e., by $(-dh/dl)/n = \sigma h$. Saturation is obtained when each molecule in the solution is excited at least once, i.e., when $\sigma h > 1$. This condition is satisfied in our laser photolysis experiments for which, with $h \simeq 10^{19}$ photons/cm^2 and $\sigma \simeq 2 \times 10^{-16}$ ($\epsilon \simeq 4 \times 10^4$), we obtain: $\sigma h \simeq 2 \times 10^3$. Thus, each RSBH$^+$ in the (deaerated) solution may be excited several thousand times during the $\sim 10^8$ s laser pulse, giving rise to detectable triplet yields even if $k_{ISC} \ll k_f$ (e.g., if $K_{ISC}/(k_{ISC} + k_f + k_d) < 10^{-3}$ as suggested by Fisher and Weiss[14]).

Less clear is the situation prevailing in O_2-saturated solutions in which the triplet yield appears to be markedly decreased. In view of the ultrafast decay of $^1RSBH^{+*}$ no dynamic (homogeneous) interactions between oxygen and the excited state are feasible. It is thus indicated that ground-state aggregation of RSBH$^+$ (or LRSBH$^+$) and O_2 leads to an excited-state $^1[RSBH^+O_2]^*$ in which the rate constant ratio $k_{ISC}/(k_f + k_d)$ is substantially reduced relative to its value in $^1RSBH^{+*}$. At present we have no independent evidence (e.g., from optical spectroscopy) as to the presence of such aggregates in O_2-saturated solutions of protonated Schiff bases of retinals. (We note, however, that "contact" CT complexes with O_2 are known for a variety of organic molecules such as amines and aromatics.[35])

RSBH$^+$/I and LRSBH$^+$/I. We finally wish to consider the phototransients RSBH$^+$/I and LRSBH$^+$/I observed in the O_2-saturated solutions of the respective systems. If, as discussed above, O_2 blocks the ISC route (K_{ISC}), an alternative low-yield pathway leading to RSBH$^+$/I (or LRSBH$^+$/I) may become detectable. A plausible identification of the latter species is one which assumes isomerization about a single bond. This would be in keeping with the "normal" values measured for the preexponential factor A and with the relatively low E_a parameter (4–7 Kcal/mol) as given in Table II.

We have also considered an alternative assignment of the above phototransients, based on the assumption that the C=C trans ↔ cis photoreactions of RSBH$^+$ and LRSBH$^+$[2] may result in photoisomers (i.e., RSBH$^+$/I and LRSBH$^+$/I) characterized by an unrelaxed Schiff base environment. This applies to both Schiff base–solvent or Schiff base–counterion interactions. (Note that such perturbations are invoked to account for the spectral shifts and for energy storage in the primary red-shifted photoproducts of bR and of visual pigments.[1]) However, this second alternative may be excluded on the basis of several arguments: First, there appears to be no correlation between solvent relaxation rates (k_r) and the decay rates (k) of RSBH$^+$/I and LRSBH$^+$/I; e.g., in n-propanol at −70 °C, −78 °C, and −90 °C, $1/k_r$ values are 2.4×10^{-8}, 6.2×10^{-8}, and 1.5×10^{-7} s, respectively, while the corresponding decay times for RSBH$^+$/I are 6×10^{-6}, 9×10^{-6}, and 1.6×10^{-5} s. Second, as shown in Table II, k in a nonpolar solvent such as CH_2Cl_2 is similar to the value measured in a "leveling solvent" such as methanol in which Schiff base–counterion interactions are negligible.[15] Moreover, the value of k in

(33) (a) Cundall, R. B. In *Progress in Reaction Kinetics*; Porter, G., Ed.; Pergamon Press: 1964; Vol. 2, pp 166–215. (b) Saltiel, J.; Charlton, J. L. Cis-Trans Isomerization of Olefins. In *Rearrangements in Ground and Excited States*; Academic Press: 1980; Vol. 3, pp 25–89. (c) Goodman, J.; Peteers, K.; Misawa, H.; Caldwell, R. J. *J. Am. Chem. Soc.* **1986**, *108*, 6803. (d) Saltiel, J.; Ganaphathy, S.; Werking, C. *J. Phys. Chem.* **1987**, *91*, 2755.

(34) Ottolenghi, M. In *Methods in Enzymology, Biomembranes P.I. Visual Pigments and Purple Membranes, II*; Packer, L., Ed.; Adacemic Press: 1982; pp 470–491.

CH_2Cl_2 is essentially insensitive to fixation of the counterion (as in the case of **6**), to the nature (size) of the counterion, and (in both CH_2Cl_2 and methanol) to the bulkiness of the amine residue, as in the cases of **7** and **8**. Finally, a relaxation mechanism based on Schiff base environmental interactions is expected to be independent of the nature of the polyene, in variance with the observed differences in k between $RSBH^+$ and $LRSBH^+$.

The suggestion that the thermal relaxations $RSBH^+/I \rightarrow RSBH^+$ (and $LRSBH^+/I \rightarrow LRSBH^+$) involve rotations around a single C–C polyene bond should be analyzed in terms of temperature and viscosity effects on k. For this purpose we adopt a well-established empirical form[35] for the (isomerization) rate constant, k_i, which applies within a family of homologous solvents (e.g., alcohols, alkanes, etc.)

$$k_i = F(\eta) \exp(-E_0/RT)$$

where $F(\eta)$ is a universal function of viscosity, and E_0 is the intrinsic molecular barrier height. The function $F(\sigma)$ assumes the empirical form[35a] $F(\sigma) = A'/\eta^a$ where A' and a are constants. As shown in the case of $LRSBH^+$ the isoviscosity plots of Figure 8 lead to a value of $E_0 = 5.5 \pm 0.4$, while Figure 9 leads to $a = 0.20 \pm 0.04$. For (double) bond (excited or ground-state) systems the parameter a is generally found[35b] to be in the range between ~ 1 (for very low isomerization barriers, when E_0 is of the order of <1 Kcal/mol) and ~ 0.1 (for higher barriers, when $E_0 > 10$ Kcal/mol). In keeping with the latter values are the essentially viscosity-independent decay rates of Cy^{III}/L for which we estimate $a < 0.5$. (No viscosity effects on the decay rate of Cy^{III}/S could be measured due to the failure to observe the latter phototransient in viscous solutions.) However, the value of $a = 0.20 \pm 0.04$ measured for $LRSBH^+/I$ appears to be somehow lower than those (0.55 ± 0.15) observed for several characteristic isomerizations with comparable E_0 values.[35b] It is possible that the weaker viscosity effect on C–C rotations in the cases of $RSBH^+$ and $LRSBH^+$ may be associated with their longer polyene chain (5 single bonds). This may induce a higher chain flexibility which enables R_iC_i–C_jR_j rotations with a relatively small displacement of the R_iC_i and C_jR_j moieties. We note in this respect that a similar concept ("bicycle pedal" model) has been invoked to account for the efficient photoisomerization of the 11-cis retinal double bond in the tightly packed binding site of visual pigments.[36]

Conclusions

In deaerated solutions of protonated retinal Schiff bases low-yield photoinduced phenomena are observed which are assigned to intersystem crossing to the triplet manifold. Under similar excitation conditions triplet states are undetectable in rhodopsins. It therefore appears that in the pigments ISC yields are further decreased, either by an enhanced $S_1 \rightarrow S_0$ rate or by a reduced $S_1 \rightarrow T$ rate. When intersystem crossing is suppressed by interaction with molecular oxygen, rotations about essential single bonds are observed. In principle, it is not unlikely that analogous rotations may play a role in the photocycles of rhodopsins contributing to the spectral shifts of photocycle intermediates. We note, however, that resonance-Raman spectroscopy[37a] and work with artificial pigments[10a,b] are not indicative of single bond rotations in the K, L, and N intermediates in the bR photocycle.[37a] The same applies to bathorhodopsin in the visual system.[37b] Thus, at present, we tend to conclude that in the protein binding site single bond isomerizations are even less effective than in the model protonated Schiff bases.

As in the case of the model Cy^{III} compound in solution, the red-shift in bR_{cy}/I is assigned to the intrinsic isomerization process, without being masked by changes in polyene–opsin interactions, such as with nonconjugated protein charges or those involving H-bonds. Thus, artificial pigments based on cyanine analogues of retinals may serve as powerful tools for investigating cis–trans isomerization reactions in the protein binding sites of visual rhodopsins and bacteriorhodopsin. In the present investigation with bR_{cy}, the results point at an abnormally low preexponential factor for the back (thermal) isomerization process. If applicable to rhodopsins this may bear on the inefficiency of the back (thermal)-reaction (from the bathorhodopsin intermediate) in the visual photocycle.

Experimental Section

Materials. 13-*cis*-Retinal was obtained from Hoffman–LaRoche. Solvents were dried and kept over 4A molecular sieves. The cyanine dye was prepared as previously described.[11]

Synthesis of the 1,1-Didemethylretinal Analogue. 1-Formyl-2-methylcyclohexene was prepared from 2-formylcyclohexanone[38] according to Harding:[39] ^1H NMR δ 1.61 (m, 4, 4-H, 5-H), 2.13 (s, 3, CH_3), 2.20 (m, 4, 3-H, 6-H), 10.14 (s, 1, CHO).

Triene Ester 10. Aldehyde **9** (150 mg, 1.2 mmol) was reacted with the sodium salt of triethyl 3-methyl-4-phosphonocrotonoate (500 mg, 2 mmol) in 25 mL of dry THF at 25 °C under argon atmosphere. After 1 h, water was added, and the mixture was extracted twice with ether. Usual workup and chromatography with ether–hexane (10:90) gave triene ester **10** (165 mg, 58% yield) as a mixture of isomers, (all-trans and 9-cis): UV (pentane) λ_{max} 310 nm (ϵ 18 000); ^1H NMR δ (all-trans) 1.28 (t, J = 7.1, 3, ester CH_3), 1.62 (m, 4, 2-H, 3-H), 1.85 (s, 3, 18-H), 2.1 (m, 4, 1-H, 4-H), 2.34 (s, 3, 19-H), 4.17 (q, J = 7.1, 2, OCH_2), 5.78 (s, 1, 10-H), 6.18 (d, J = 16.5, 1, 8-H), 7.10 (d, J = 16.5, 1, 7-H); high resolution MS ($C_{15}H_{22}O_2$) calcd 234.1619, found 234.1562, 161.1360 (M – CO_2Et), 146.1095 (M – CO_2Et – CH_3), 121.1046 (M – CH_3CCHCO_2Et), 95.0891 (methylcyclohexene)$^+$.

Trienol 11. Triene ester **10** (165 mg, 0.7 mmol) was dissolved in 6 mL of dry THF under argon atmosphere. The solution was cooled to −78 °C, and 2 mL of a 1 M hexane solution of diisobutylaluminum hydride was added. After 2 h at −78 °C the reaction was quenched with ethyl acetate (2 mL), and the temperature was raised gradually to 25 °C followed by addition of water (2 mL). The mixture was stirred for 15 min and filtered through Celite. Evaporation of the solvent gave crude trienol **11** (135 mg) which was directly oxidized, without further purification: ^1H NMR δ 1.61 (m, 4, 2-H, 3-H), 1.81 (s, 3, 19-H), 2.10 (m, 4, 1-H, 4-H), 4.28 (d, J = 7.0, 2, 11-H), 5.66 (t, J = 7.0, 1, 10-H), 6.18 (d, J = 16.0, 1, 8-H), 6.74 (d, J = 16.0, 1, 7-H).

Trienal 12. Trienol **11** (135 mg, 0.7 mmol) was dissolved in 20 mL of methylene chloride. Manganese dioxide (270 mg) was added, and the reaction mixture was stirred at 25 °C for 18 h, followed by filtration through Celite and solvent evaporation. Chromatography with ether–hexane (10:90) gave trienal **12** (80 mg, 60% yield), in a 7:3 trans–cis ratio: UV (ethanol) λ_{max} 338 nm (ϵ, 25 000); ^1H NMR δ (all-trans) 1.64 (m, 4, 2-H, 3-H), 1.89 (s, 3, 18-H), 2.17 (m, 4, 1-H, 4-H), 2.32 (s, 3, 19-H), 5.98 (d, J = 8.2, 1, 10-H), 6.26 (d, J = 15.8, 1, 8-H), 7.26 (d, J = 15.8, 1, 7-H), 10.10 (d, J = 8.2, 1, 11-H); (9-cis) 1.65 (m, 4, 2-H, 3-H), 1.87 (s, 3, 18-H), 2.13 (s, 3, 19-H), 2.14 (m, 4, 1-H, 4-H), 5.84 (d, J = 7.9, 1, 10-H), 7.16 (s, 2, 7-H, 8-H), 10.20 (d, J = 7.9, 1, 11-H); high resolution MS ($C_{13}H_{18}O$) calcd 190.1358, found 190.1320, 175.1102 (M – CH_2), 161.1021 (M – CHO).

Pentaenenitrile 13, *all-trans*-Trienal **12** (75 mg, 0.4 mmol) was reacted with the sodium salt of diethyl 3-methyl-4-phosphonocrotononitrile (200 mg, 0.93 mmol) in 15 mL of dry THF at 25 °C under argon atmosphere. After 15 min, water was added, and the mixture was extracted twice with ether. Usual workup and chromatography with ether–hexane (10:90) gave pentaenenitrile **13** (99 mg, 99% yield) as a mixture of isomers: UV (pentane) λ_{max} 369 nm (ϵ, 35 000); ^1H NMR δ (all-trans) 1.61 (m, 4, 2-H, 3-H), 1.84 (s, 3, 18-H), 2.01 (s, 3, 19-H), 2.14 (m, 4, 1-H, 4-H), 2.20 (s, 3, 20-H), 5.16 (s, 1, 14-H), 6.16 (d, J = 11.4, 1, 10-H), 6.25 (d, J = 15, 2, 8-H, 12-H), 6.89 (d, J = 15, 1, 7-H), 6.96 (dd, J_1 = 11.4, J_2 = 15.1, 1, 11-H); high resolution MS ($C_{18}H_{23}N$) calcd 253.1830, found 253.1821, 238.1621 (M – CH_2), 158.0966 (M – methylcyclohexene).

1,1-Didemethylretinal (14). Pentaenenitrile **13** (90 mg, 0.36 mmol) was dissolved in 10 mL of dry hexane under argon atmosphere. The solution was cooled to −78 °C and stirred with 1 mL of a 1 M hexane solution of diisobutylaluminum hydride. After 2 h at −78 °C, ether (10 ml) and wet silica (1.5 g) were added, and the mixture was stirred for 2 h at 4 °C followed by filtration through Celite and solvent evaporation.

(35) (a) Gegiou, G.; Muzkat, K. A.; Fisher, E. *J. Am. Chem. Soc.* **1968**, *90*, 12. (b) Fleming, G. R.; Waldeck, D. H.; Keery, K. M.; Velsko, S. P. In *Applications of Picosecond Spectroscopy to Chemistry*; Eisenthal, K. B., Ed.; Riedel Publishing Co.: 1984; pp 67–77.

(36) Warshel, A. *Nature (London)* **1976**, *260*, 679.

(37) (a) Fodor, S.; Pollard, W.; Gebhard, R.; Van der Berg, E.; Lugtenburg, J.; Mathies, R. *Proc. Natl. Acad. Sci. U.S.A.*, in press. (b) Palings, I.; Pardoen, J.; Van der Berg, E.; Winkel, C.; Lugtenburg, J.; Mathies, R. *Biochemistry* **1987**, *26*, 2544.

(38) Ainsworth, C. *Organic Synthesis*; Wiley: New York, Collect. Vol. 4, p 536.

(39) Harding, K. E.; Ligon, R. C. *Synth. Commun.* **1974**, *4*, 297.

Chromatography with ether-hexane (10:90) gave 1,1-didemethylretinal (86 mg, 94% yield) in a 2:1 all-trans:13-cis ratio: UV (pentane) λ_{max} 384 nm (ϵ 23 200) (all-trans), 378 nm (ϵ, 18 000) (13-cis); ^{1}H NMR δ (all-trans) 1.63 (m, 4, 2-H, 3-H), 1.86 (s, 3, 18-H), 2.05 (s, 3, 19-H), 2.1-2.2 (m, 4, 1-H, 4-H), 2.3 (s, 3, 20-H), 5.98 (d, J = 8.1, 1, 14-H), 6.26 (d, J = 11.6, 1, 10-H), 6.30 (d, J = 15.7, 1, 8-H), 6.38 (d, J = 14.9, 1, 12-H), 6.91 (d, J = 15.7, 1, 7-H), 7.16 (dd, J_1 = 11.6, J_2 = 14.9, 1, 11-H), 10.10 (d, J = 8.1, 1, 15-H); (13-cis) 1.63 (m, 4, 2-H, 3-H), 1.86 (s, 3, 18-H), 2.05 (s, 3, 19-H), 2.1-2.2 (m, 4, 1-H, 4-H), 2.16 (s, 3, 20-H), 5.84 (d, J = 8.0, 1, 14-H), 6.31 (d, J = 11.6, 1, 10-H), 6.32 (d, J = 15.7, 1, 8-H), 6.91 (d, J = 15.7, 1, 7-H), 7.05 (dd, J_1 = 11.6, J_2 = 14.9, 1, 11-H), 7.28 (d, J = 14.9, 1, 12-H), 10.22 (d, J = 8.0, 1, 15-H); CI-MS 257 (MH^+), 239 (MH - H_2O), 161 (MH - methylcyclohexene).

The Schiff bases were prepared by dissolving the aldehyde in dry ethanol followed by addition of the corresponding amine (1.5 equiv) at 25 °C. The reaction mixture was stirred for 30 min followed by evaporation of the ethanol and excess of amine under high vacuum. The Schiff bases were dissolved in the required solvent and were protonated with solution saturated with HCl. Immonium salt **5** was prepared by mixing (1.2 equiv) pyrrolidine perchlorate with the corresponding aldehyde in ethanol for 3 h at 25 °C. The ethanol was evaporated under high vacuum, and the residual oil was dissolved in the required solvent. Immonium salt **6** was prepared similarly by mixing the aldehyde with proline in trifluorethanol for 10 h at 25 °C.

Laser Photolysis. Pulsed-laser photolysis experiments were carried out with a UV-14, DL-200 Molectron N_2/dye laser system (8 ns, 0.5-1 mJ). Use was also made (especially for the low-temperature measurements) of a Nd:YAG dye laser system (15 ms, 10-50 mJ) equipped with an appropriate cryostate. Actual exciting light intensities (mJ/cm^2) were also controlled by the use of lenses and netural density filters. Data were digitized with Biomation 8100 or Tektronix 7912 transient recorders, followed by computer averaging and analysis.

Acknowledgment. Work supported by the U.S.-Israel Binational Science Foundation, by the Fund for Basic Research (administrated by the Israeli Academies of Sciences and Humanities), by the E. D. Bergman H.U. Fund, and by the Yeda Fund. M.S. is an incumbent of the Morris and Ida Wolf Career Development Chair.

Deuterium Quadrupole Echo Study of Urea Motion in Urea/*n*-Alkane Inclusion Compounds

N. J. Heaton, R. L. Vold, and R. R. Vold*

Contribution from the Department of Chemistry, University of California—San Diego, La Jolla, California 92093. *Received September 15, 1988*

Abstract: Urea molecules constituting the host lattice of the inclusion compound, urea-d_4/*n*-nonadecane, have been found to undergo 180° flips about the C=O axis at the rate of 2.0×10^6 s^{-1} at 303 K with an activation energy of 23 ± 2 kJ mol^{-1}. The kinetic parameters for this process were obtained from simulations of the deuterium quadrupole echo spectra at a pulse spacing at 40 μs. At longer pulse spacings the quadrupole echo line shapes reveal the presence of an additional dephasing process which is angle dependent and can be ascribed to unresolved dipolar interactions, in particular, the nitrogen-deuterium coupling.

Urea forms adducts with a variety of organic compounds such as unbranched alcohols, acids, and *n*-alkanes. In all cases, spirals of hydrogen-bonded urea molecules form the walls of hexagonal channels within which the guest molecules reside in extended conformations and undergo rapid rotation about their long axis. The urea molecules lie almost coplanar with the channel walls, with the carbonyl bonds oriented at 90° to the long axes of the channels. Although the structures are nonstoichiometric with respect to the urea/guest ratio, in almost all cases the repeat distance of the urea channel structure along the crystal Z axis is 11 Å and the channel diameter is about 5 Å.[1-3] The guest molecules exhibit considerable disorder along the crystal Z axis while being highly ordered in directions perpendicular to this axis.[4] At room temperature the unit cell of the channel structure is hexagonal and contains six urea molecules. The dimensions of the channels are such that they are able to accomodate only unbranched aliphatic compounds. On heating, the inclusion compounds decompose to give pure urea and the guest compound while at low temperatures (~160 K for $C_{19}H_{40}$ adduct) the complexes undergo a phase transition from the hexagonal to an orthorhombic structure. Both the thermal stability and the phase transition temperature vary significantly with the length of the guest molecule.[5-9] A comprehensive review of the properties and applications of urea inclusion compounds is available.[10]

The conformational and dynamic properties of these compounds have been investigated by several groups using ^{1}H,[11] ^{13}C,[12-14] and ^{2}H[15-17] NMR techniques. Most of the studies to date have been concerned only with the dynamics of the guest molecules while the channel walls themselves have generally been regarded as rigid. The only reported studies of channel dynamics in urea inclusion compounds are those of Clement et al.[18,19] They observed that the lines in the ^{14}N NQR spectrum of the urea/trioxane complex disappeared as the temperature was raised above 283 K and proposed that this might be the result of hindered rotation of the urea molecules about their C=O bonds although they did not characterize the nature of the motion in any detail. It would be unwise, however, to draw general conclusions concerning channel motion in urea adducts from these results since the urea/trioxane

(1) Smith, A. E. *Acta Crystallogr.* **1952**, *5*, 224.
(2) Lenne, H. *Acta Crystallogr.* **1954**, *7*, 1.
(3) Schlenk, W. *Justus Liebigs Ann. Chem.* **1949**, *565*, 204.
(4) Fukao, K.; Myaji, H.; Asai, K. *J. Chem. Phys.* **1986**, *84*, 6360.
(5) Chatani, Y.; Anraku, H.; Taki, Y. *Mol. Cryst. Liquid Cryst.* **197**, *48*, 219.
(6) Pemberton, R. C.; Parsonage, N. G. *Trans. Faraday Soc.* **1965**, *61*, 2112.
(7) Pemberton, R. C.; Parsonage, N. G. *Trans. Faraday Soc.* **1966**, *62*, 553.
(8) Pemberton, R. C.; Parsonage, N. G. *Trans. Faraday Soc.* **1967**, *63*, 311.
(9) McAdie, H. G. *Can. J. Chem.* **1962**, *40*, 2195.
(10) Takemoto, K.; Sonoda, N. In *Inclusion Compounds* Attwood, J. L., Davies, J. E., MacNicol, D. D., Eds.; Academic Press: Orlando, FL, 1984; Vol. 2.
(11) Gilson, D. F. R.; McDowell, C. A. *Mol. Phys.* **1961**, *4*, 125.
(12) Imashiro, F.; Maeda, T.; Nakai, T.; Saika, A.; Terao, T. *J. Phys. Chem.* **1986**, *90*, 5498.
(13) Okazaki, M.; Naito, A.; McDowell, C. A. *Chem. Phys. Lett.* **1983**, *100*, 15.
(14) Okazaki, M.; McDowell, C. A. *J. Mol. Struct.* **1984**, *118*, 149.
(15) Meirovitch, E.; Belsky, I. *J. Phys. Chem.* **1984**, *88*, 6407.
(16) Casal, H. L.; Cameron, D. G.; Kelusky, E. *J. Chem. Phys.* **1984**, *80*, 1407.
(17) Greenfield, M. S.; Vold, R. L.; Vold, R. R. *J. Chem. Phys.* **1985**, *83*, 1440.
(18) Clement, R.; Mazieres, C.; Guibe, L. *J. Solid State Chem.* **1972**, *5*, 436.
(19) Clement, R.; Gourdji, M.; Guibe, L. *J. Magn. Reson.* **1975**, *20*, 345.

0002-7863/89/1511-3211$01.50/0