

Schiff Base of 11-*cis*-Retinal: Photoisomerization as a Function of Solvent and Protonation

Ralph S. Becker,* Kenn Freedman, and Guy Causey

Department of Chemistry, University of Houston-Central
Houston, Texas 77004

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Until now, the *n*-butylamine Schiff base of retinal (RSB) has been studied as a mimic of the visual pigment rhodopsin. The quantum yield of photoisomerization (ϕ_{PI}) of 11-*cis*-RSB in hexane with assumption of isomerization to *all-trans*-RSB has been reported to be quite small but nonetheless finite¹ ($\phi_{PI} 4 \times 10^{-3}$). In the case of protonated 11-*cis*-RSB in a methanol solvent, again with assumption of isomerization to *all-trans*, the ϕ_{PI} was reported to be potentially much larger (0.005–0.25, excitation wavelength dependent).² There does not appear to be any other published work regarding the ϕ_{PI} of these important mimic molecules.

As part of our continuing study of polyenes and the visual process, we have studied the photoisomerization of the 11-*cis*-RSB and -RSBH⁺ in the polar solvents dichloromethane, acetonitrile, and methanol and in the alkane solvents 3-methylpentane (3MP) and hexane.

ϕ_{PI} data were determined for the 11-*cis* isomer by a comparison method,³ using benzophenone in benzene ($\epsilon_{TT}^{532.5} 7630$, $\phi_T 1.0$) as a reference and utilizing $\Delta\epsilon_{SS}$ data obtained from absorption spectra of 11-*cis* and *all-trans* isomers in the appropriate solvent ($\Delta\epsilon_{SS}$ is the difference in the extinction coefficients). ϕ_{PI} reported were an average of at least three independent samples. The Schiff bases were prepared as before⁴ and protonated with trichloroacetic acid. The excitation source was the 337-nm line of a N₂ laser (or the 355-nm line of a Nd:YAG laser). ϕ_{PI} did not depend on these wavelength differences. The laser flash equipment used has been described previously.^{5,6}

In the polar solvents, positive changes in the optical density (ΔOD) were observed over the wavelength regions chosen for both the protonated (400–500 nm) and unprotonated (350–450 nm) RSB's. The ΔOD spectra for the RSB and RSBH⁺ in dichloromethane are shown in Figure 1. Similar spectra were obtained for the RSB and RSBH⁺ in acetonitrile and methanol. No decay of these spectra occurred up to 400 μ s. In addition, no triplet transients or other comparable short-lived species were observed.

Substantiation of the fact that the primary process was isomerization of 11-*cis*-RSB to *all-trans*-RSB was accomplished as follows. A solution of 11-*cis*-RSB ($\sim 10^{-5}$ M) was irradiated in a Cary spectrophotometer, employing a filter with maximum transmission at 365 nm, for successive time periods beginning with 20 s and for a total of 3 min. The final spectra showed an almost complete loss of the *cis* band at ~ 250 nm, a slight red shift in the long-wavelength band, and a substantial increase in the OD, generating spectra typical of known *all-trans*-RSB. Second, even after 20 s, TLC of the solution showed only the presence of *all-trans*- and 11-*cis*-RSB's. Prior to this, the R_f values of 11-*cis*- (0.66) and *all-trans*-RSB (0.48) were established by using ether as the eluting solvent on silica gel plates. In addition, a mixture of known pure 11-*cis*- and *all-trans*-RSB's showed complete separation in TLC with R_f values the same as for the separate

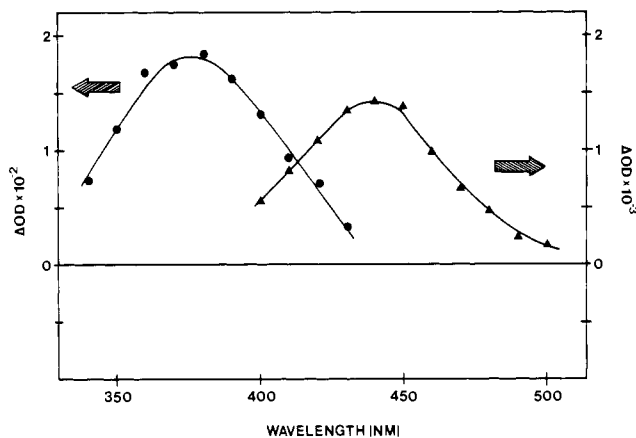


Figure 1. Typical ΔOD vs. wavelength plots for RSB (●) and RSBH⁺ (▲) in CH₂Cl₂ recorded up to 400 μ s after 337-nm laser flash (one laser pulse per data point; resolution of 10 nm).

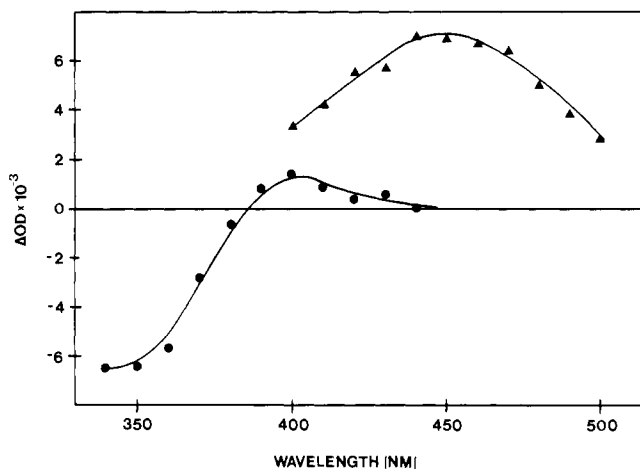


Figure 2. ΔOD vs. wavelength plots for RSB (●) and RSBH⁺ (▲) in 3MP.

Table I. Photoisomerization Yields (ϕ_{PI}) to *All-Trans* Isomers

solvent	11- <i>cis</i> -RSB	11- <i>cis</i> -RSBH ⁺
3MP, hexane	<i>a</i>	0.17 \pm 0.03
methanol	0.07 \pm .01	0.07 \pm 0.01
acetonitrile	0.09 \pm .02	0.13 \pm 0.01
dichloromethane	0.14 \pm .02	0.14 \pm 0.04

^a Isomerization did not occur to *all-trans* (see text).

pure compounds. Finally in separate experiments, after 15–20 laser pulses, the typical number needed to generate spectra as in Figure 1, TLC of the solution showed only *all-trans*- and 11-*cis*-RSB's.

For protonated 11-*cis*-RSB, after Hg lamp or laser irradiation, triethylamine was added to neutralize the trichloroacetic acid before TLC.⁷ Again only *all-trans*- and 11-*cis*-RSB's were present for each solvent examined. Separate experiments employing the same neutralization technique on samples that were not irradiated did not yield *all-trans*-RSB.

The quantum yields of photoisomerization in the polar solvents are listed in Table I. The ϕ_{PI} and the lack of a transient are independent of the presence of pure O₂ or N₂.

For the alkane solvents, 3MP and hexane, surprising differences were observed compared to the other solvents for unprotonated 11-*cis*-RSB. Over a significant wavelength range, notable negative ΔOD 's occurred (Figure 2). On the basis of the difference in the $\Delta\epsilon_{SS}$ of 11-*cis*- and *all-trans*-RSB's at room temperature, positive ΔOD 's must occur if an 11-*cis* to *all-trans* isomerization

(7) We thank Peter Wagner for this suggestion of neutralization since TLC of the protonated species did not give good resolution.

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(6) The laser flash spectroscopy was performed at the Center for Fast Kinetics Research at the University of Texas at Austin, which is supported by NIH Grant RR-00886, the Biotechnology Branch of the Division of Research Resources, and the University of Texas. This research was supported by funds of one of the authors (R.S.B.) and a Biomedical Research Support Grant. We thank Hoffman-La Roche Inc. for their kind gift of 11-*cis*-retinal.

takes place (slightly positive ΔOD 's were seen only after ~ 390 nm). These results are inconsistent with an 11-12-cis to 11-12-trans isomerization as assumed earlier.¹ TLC of either Hg lamp (5 min) or laser (15 pulses) irradiated samples did not show the presence of *all-trans*-RSB (the only definite spot corresponded to 11-*cis*-RSB). Therefore we propose that the ΔOD spectrum that was consistently observed corresponds to another isomerization process such as a trans to cis isomerization about another double bond with a very low ϕ_{PI} .

In the case of protonated 11-*cis*-RSB in alkanes, positive ΔOD 's were observed over the entire wavelength region (400–500 nm) studied (Figure 2). Identification of *all-trans*-RSB as the primary photoproduct was accomplished by TLC as above except 20% hexane–80% ether was used as the eluting solvent. The ϕ_{PI} values are listed in Table I.

It appears that in an alkane solvent, the absence of a proton not only prohibits the 11-12-cis to 11-12-trans isomerization but may promote another type of isomerization. The proton in this type of solvent (alkane) does play a role in directing the same type of isomerization that occurs in rhodopsin. However, in the case of the polar solvents, protonation exerts no influence on the type of isomerization already existing for the unprotonated RSB and little or no influence on the ϕ_{PI} . This clearly indicates that protonation is not a general requirement for the 11-cis to all-trans isomerization in the Schiff base.

These results raise important questions regarding a restricted view of the proton as necessary for promoting the isomerization of rhodopsin and open the way for broader roles of the proton in the early steps of the visual process.

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Electron-Transfer Kinetics of Pentaammineruthenium(III)(histidine-33)-Ferricytochrome *c*. Measurement of the Rate of Intramolecular Electron Transfer between Redox Centers Separated by 15 Å in a Protein

Jay R. Winkler, Daniel G. Nocera, Kathryn M. Yocom, Emilio Bordignon,¹ and Harry B. Gray*

Contribution No. 6637 from the Arthur Amos Noyes Laboratory California Institute of Technology Pasadena, California 91125

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A central problem in metalloprotein oxidation-reduction chemistry is the elucidation of the relationship between electron-transfer rate and redox-center separation.²⁻⁴ The most direct way to address this problem is to make measurements of intramolecular electron-transfer rates between redox centers in proteins in cases where the separation distance is known. An attractive system in this regard is $Ru(NH_3)_5(His-33)^{3+}$ -ferricytochrome *c* ($PFe^{III}-Ru^{III}$), whose redox centers are separated by 15 Å (Figure 1).^{4b} This protein derivative is ideally suited for electron-transfer kinetic studies, because the $Ru(NH_3)_5(His-33)^{n+}$ unit is substitution inert in both $n = 3$ and $n = 2$ oxidation states and its reduction potential is very near that of the heme *c* ($PFe^{III}-Ru^{III}$; $E^\circ Fe^{III}/Fe^{II} = 0.26$ V vs. NHE; $E^\circ(Ru^{III}/Ru^{II}) = 0.15$ V vs.

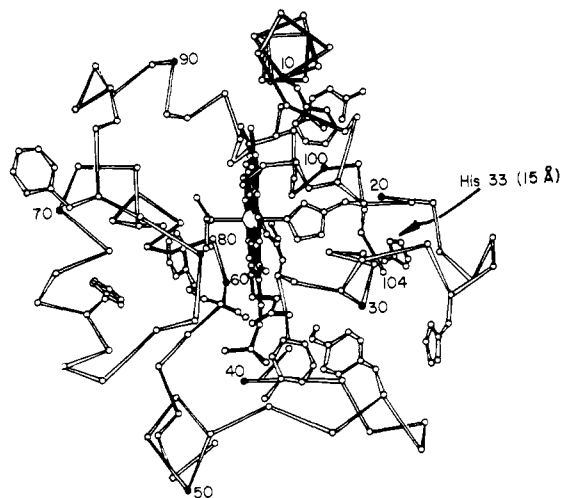


Figure 1. Molecular skeleton of horse heart cytochrome *c* illustrating the position of His-33 relative to the heme *c* group (the axial Fe ligands are Met-80 and His-18). The shortest (His-33)-(heme *c*) distance is 15 (1) Å (imidazole N3 to the closest aromatic C in the porphyrin is shorter than any of the imidazole(His-33)-imidazole(His-18) contacts).^{4b}

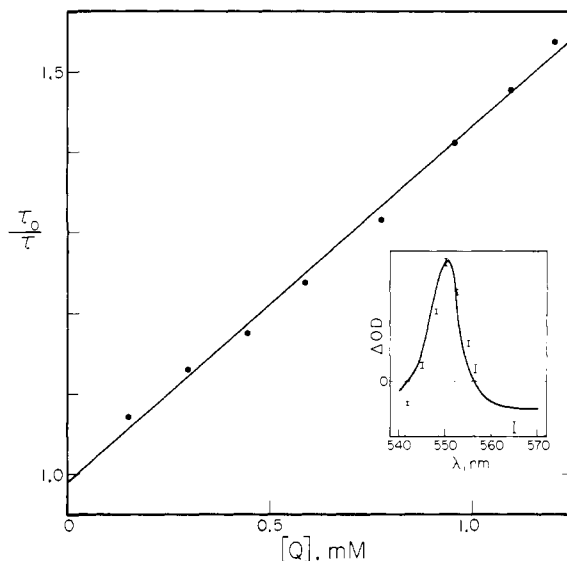


Figure 2. Stern-Volmer plot of luminescence quenching of $Ru(bpy)_3^{2+}$ ($\tau_0 = 570$ ns) by horse heart $PFe^{III}-Ru^{III}$ in 0.1μ (pH 7) phosphate buffer under nitrogen. Excitation source: second harmonic (532 nm), Nd:YAG laser (pulse duration, 8 ns fwhm). Inset: solid line, transient difference spectrum calculated from the absorption spectra of PFe^{III} and PFe^{II} ; points with error bars, transient difference spectrum for a $PFe^{III}/Ru(bpy)_3^{2+}$ solution.

NHE). Here we report experiments that show unambiguously that electron transfer from Ru^{II} to Fe^{III} in the "mixed-valence" $PFe^{III}-Ru^{II}$ ($d = 15 \text{ Å}$) species takes place at a significant rate.

The long-lived excited state of $Ru(bpy)_3^{2+}$ ($bpy = 2,2'$ -bipyridine) is a powerful reducing agent,⁵ and we have demonstrated⁶ that it will transfer electrons to both PFe^{III} and $Ru(NH_3)_5His^{3+}$. A Stern-Volmer plot of the luminescence quenching of $Ru(bpy)_3^{2+}$ by $PFe^{III}-Ru^{III}$ (Figure 2) yields $k_q(PFe^{III}-Ru^{III}) = 7.8 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. The inset in Figure 2 is the transient absorption spectrum of a deaerated solution of $Ru(bpy)_3^{2+}$ and PFe^{III} recorded

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(6) The rate constants for luminescence quenching of $Ru(bpy)_3^{2+}$ under a nitrogen atmosphere in 0.1μ (pH 7) phosphate buffer by purified horse heart PFe^{III} and $Ru(NH_3)_5His^{3+}$ are 2.5×10^8 and $1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, respectively. Quenching of $Ru(bpy)_3^{2+}$ by PFe^{III} has been studied previously (Sutin, N. *Adv. Chem. Ser.* 1977, No. 162, 156. McLendon, G.; Lum, V. R.; English, A. M. Gray, H. B., unpublished results).