Visual Pigments. 7. Experimental and Theoretical Investigations of the Absorption Spectral Properties of Protonated Retinal Schiff Bases and Implications for the Bathochromic Shift in Visual Pigments[1]

Walter H. Waddell, Arnold M. Schaffer, and Ralph S. Becker*[2]

Contribution from the Department of Chemistry, University of Houston, Houston, Texas 77004. *Received January 28, 1977*

Abstract: The room (295 K) and low (77 K) temperature absorption spectral properties of the protonated all-trans methyl and the all-trans and 11-cis *n*-butyl retinal Schiff bases have been examined. Upon addition of excessive amounts of anhydrous HCl (or HBr) gas to a solution of the Schiff base in 3-methylpentane and immediate cooling to 77 K, new or shifted in position bands maximizing at ca. 540 and 318 nm can be observed. With the utilization of predicted band maxima from semiempirical theoretical calculations, we conclude that the new or shifted bands result from a lowering of the transition energy of the protonated Schiff base as a result of the preferential lowering of the excited singlet state (relative to the ground state) of the polyene because of interaction with the hydrogen halide solvent cage formed under our experimental conditions. These experimental results are consistent with the suggestion that in rhodopsin the lowering in energy of the chromophore's transition occurs via secondary noncovalent interactions with the protein opsin. The low-energy transition of rhodopsin, its bleaching intermediates, and synthetic pigments are briefly discussed.

Introduction

In their pioneering studies of the visual pigment rhodopsin, Wald and Hubbard[3] determined that 11-cis retinal was the chromophore bound to the protein opsin. The early suggestion by Collins[4] and Morton and Pitt[5] that the primary binding of 11-cis retinal to opsin was a protonated Schiff base linkage has recently received direct support from resonance Raman studies.[6-9] It is generally accepted that the chromophore is bound covalently to a ϵ-amino group of a lysine in opsin.[10-13]

Rhodopsin has a first absorption band maximum at 498 nm, ϵ_{max} 40 600 M^{-1} cm^{-1}. Protonated Schiff bases of all-trans retinal and aliphatic amines have an absorption maximum at ca. 440 nm,[15] which is at much shorter wavelengths (blue shifted) that the absorption maxima of rhodopsin, bathorhodopsin (formerly prelumirhodopsin, λ_{max} 543 nm), subsequent bleaching intermediates,[16] and the synthetic pigments isorhodopsin[3] and 9,13-isorhodopsin (isorhodopsin II).[17] This large bathochromic shift (shift to longer wavelengths; red shift) between the absorption maxima of visual pigments and simple protonated retinal Schiff bases has been the subject of much concern. Several theories have been proposed to explain the long-wavelength absorption maximum of rhodopsin.[15] Hubbard and Kropf[18,19] have suggested that the low-energy transition of the chromophore in visual pigments is the result of a stabilizing effect that the protein has upon the polyene via secondary Coulombic interactions. Blatz and co-workers[20-24] have proposed that the protein controls the transition energy of the chromophore by regulating the distance of the counteranion to the nitrogen of the protonated Schiff base linkage. Leermakers and co-workers[25-27] postulated that the bathochromic shift in the absorption maximum of rhodopsin is due to a stabilization of the transition states as a result of interactions with the microenvironment of the chromophore resulting from the polarizability of the aromatic amino acids in opsin.

Honig, Ebrey, and co-workers[28] have recently suggested that the long-wavelength absorption maximum of rhodopsin can be accounted for using a modification of the proposal by Hubbard and Kropf.[18,19] They suggest that the protonated Schiff base is in close association with its counterion and that an additional negatively charged (or polar) group(s) is located near the β-ionone ring of the chromophore in the protein.

We[1,29] have previously reported on a protonated Schiff base of all-trans retinal that had an unusually long wavelength first absorption band maximum of 543 nm at 77 K when an *excess* of hydrogen chloride gas was used as a protonating species in a hydrocarbon solvent. Since hydrogen chloride is not dissociated under these conditions, it is possible that Coulombic interactions exist between the chlorine atom and the carbon atoms of the π system of the polyene. These interactions may be of the same nature as those suggested by Hubbard and Kropf.[18,19] We have, therefore, made a detailed experimental and theoretical investigation of the absorption spectral properties of the protonated Schiff bases of all-trans and 11-cis retinal.

Experimental Section

All-trans retinal (Sigma Chemical Co.) and 11-cis retinal (a generous gift of the Hoffmann-La Roche Chemical Co.) were used without further purification. *n*-Butylamine (Fisher Scientific) was distilled prior to use. The all-trans *n*-butyl retinal Schiff base was prepared by dissolving all-trans retinal in methanol, cooling to −78 °C, adding an excess of *n*-butylamine, reacting overnight at 0 °C in the presence of a 3 A molecular sieve, filtering to remove the molecular sieve, and removing the unreacted *n*-butylamine under vacuum. The 11-cis Schiff base was prepared by dissolving the retinal in 3-methylpentane (~10^{-4} M), cooling to −78 °C, adding an equimolar amount of *n*-butylamine, and removing the solvent and unreacted *n*-butylamine under vacuum. The all-trans methyl Schiff base was prepared by bubbling monomethylamine (Matheson Gas) into a chloroform solution of retinal containing a 3 A molecular sieve, filtering, and evaporating to dryness under vacuum.

The all-trans Schiff bases were protonated at room temperature by bubbling hydrogen chloride (Matheson Gas) or hydrogen bromide (Linde Specialty Gas) directly into a sample solution contained in a 2-mm quartz cell. The 11-cis Schiff base was protonated at −63 °C using a chloroform/liquid nitrogen slush bath, since protonation at room temperature sometimes resulted in an absorption spectrum with low intensity in the 250-nm region, the "cis" peak, probably the result of some cis → trans isomerization. All gases were purchased as anhydrous and passed through subsequent drying traps of concentrated sulfuric acid, silica gel, and calcium chloride, then directly into the sample cell.

The solvent for all spectroscopic studies (unless otherwise specified) was 3-methylpentane (3MP, Phillips Petroleum Co., 99+ mol %) which was refluxed and distilled from Dri-Na (Baker Chemicals) and chromatographed on silver nitrate alumina. 2-Methyltetrahydrofuran

was distilled from calcium hydride before use.

Absorption spectra were recorded on a Cary Model 15 spectrophotometer. Flat-faced quartz cells were used in conjunction with flat-faced quartz liquid nitrogen Dewars. All optical quartz was of Suprasil II. Temperatures intermediate between 77 and 295 K were attained using a Wheelco Model 402 Capacitrol which was calibrated at 24, 0, −78, and −196 °C to be accurate within two degrees over this range. All spectra were corrected for surface reflection and background absorption.

Oscillator strengths were determined by replotting the absorption spectrum on an abscissa linear in wavenumber and an ordinate linear in molar extinction coefficient. A large reproduction of this spectrum was empirically divided into individual transitions assuming Gaussian band shapes. The numbers, which include no corrections due to the refractive index of the solvent, are measurable to within ±0.05.

Calculations

The results given in Table I for the all-trans protonated Schiff base were obtained using C=C, C-C, C=N bond distances of 1.336, 1.460, and 1.280 Å, respectively. A simple Hückel calculation used standard parameters.[30] An extended Hückel (EH) calculation followed a charge self-consistency procedure[31] with the nitrogen HCl bond distance set at 1.03 Å. All Pariser-Parr-Pople (PPP) calculations were carried out within the framework of the usual SCF-MO-CI method as previously described[32] in which all singly excited configurations up to 15 eV were included. The effect of protonation (i.e., the interaction between the imine nitrogen and HCl) was incorporated into the PPP model in one of several ways. One model assumed that the effect of protonation was to delocalize the positive charge along the polyene chain and thus alter the ionization potential and electron affinity of the charged atoms. Calculations were also made (Table I) using the electrostatic model of Mataga and Mataga[33] in which a proton of variable charge (Z_{H+}) is bound to the nitrogen lone pair by an electrostatic force. The effect of negative point charges above the polyene chain was included by altering the Coulomb integral of the carbon atoms in the π electron system. Calculations were also made assuming a covalent model[34] in which there is an electron transfer from the nitrogen to the proton, leading to covalent bonding. The CNDO calculations reported in the text were made using a program written by Dobash.[35]

Results

The room and low (77 K) temperature absorption spectral properties of the protonated all-trans methyl and the all-trans and 11-cis *n*-butyl retinal Schiff bases have been examined. We have previously made a detailed investigation of the absorption spectral properties of the isomeric Schiff bases.[32] At room temperature in 3MP solutions, the all-trans *n*-butyl Schiff base has a first absorption band maximum at 356 nm, which is shifted to 435 nm upon addition of anhydrous hydrogen chloride gas. This band is broad and structureless. Cooling this solution to 77 K results in a slight shift of the band maxima to 440 nm with little change in the band shape, Figure 1. At 77 K a second band having vibrational fine structure can be observed in the 310-360-nm region.

Alternatively, if the solution is immediately plunged into liquid nitrogen after the addition of the anhydrous HCl gas, the solution becomes purple, rather than yellow-brown, and a new absorption spectrum can be recorded. This absorption spectrum has new bands at 542, 523, 505, and 318 nm, with a shift in the 440-nm band to ca. 460 nm.[29] The intensity of the 542-nm band and the presence of the other bands is very dependent upon the concentration of HCl. To maximize its intensity, a large excess of HCl must be used and the solution should be cooled *immediately* to 77 K, even while the gas is being added. An alternative method is to warm a solution displaying the 542-nm band as a shoulder from 77 K to ca. 100 K. At this temperature, the rigid 3MP matrix melted, diffusion was visually observed to occur, and the 542- and 318-nm band intensities grew at the expense of the 460-nm band.[29]

The 542- and 318-nm bands can be observed when the solution is warmed as high as 143 K, and recooled to 77 K, but above this temperature (ca. 145 K) the room temperature spectrum (λ_{max} 435 nm) is observed and recooling this solution to 77 K does not regenerate the 542-nm band, but results in a small shift to 440 nm. A solution that did not initially have a 542-nm band (or shoulder) at 77 K was allowed to set at ca. 100 K, and the 542- and 318-nm bands were generated. No dependence on the Schiff base concentration was observed in the 10^{-4}-10^{-5} M range. Owing to the instability of this long-wavelength absorbing species, it was not possible for us to characterize it by other spectral methods.

Two results indicate that a two-species equilibrium is occurring with one species absorbing at 460 nm and the other at 542 nm (with associated bands, vide supra). If a solution that only has a shoulder at 542 nm is allowed to set at 97 K, this band increases and the 460-nm band decreases. An isobestic point is observed at 486 nm.[29] Emission studies[36] indicate that two different fluorescences can be observed upon excitation at 460 and 540 nm. Furthermore, the 318- and 542-nm bands must originate from the same species since their absorptions grow in conjunction with one another; excitation into either of these bands results in the same fluorescence, and the fluo-

Table I. Models Used for Calculating the Absorption Maximum of All-Trans Schiff Bases with HCl[a]

PPP method	Calcd max, nm
I Delocalization of Charge along Chain	
1. +1 charge on N	425
2. +0.6 on N, +0.4 on adjacent C	496
II Electrostatic Model	
1. $Z_{H^+} = 0.4$ $r_{N\text{-}H^+} = 1.03$	454
2. $Z_{H^+} = 0.8$ $r_{N\text{-}H^+} = 1.03$	543
3. $Z_{H^+} = 0.8$ $r_{N\text{-}H^+} = 1.29$	521
4. $Z_{H^+} = 0.4$ $r_{N\text{-}H^+} = 1.03$, increase conjugation along chain	496
III Electrostatic Model with Negative Point Charges above Chain	
1. (−) 3 Å above C_{15}	411
2. (−) 3 Å above C_{11}	527
3. (−) 3 Å above C_9	507
IV Covalent Model	
1. No polarization of σ core (+N)	454
2. +0.4 on C adjacent to N	503

[a] The method of calculation is described more fully in the text.

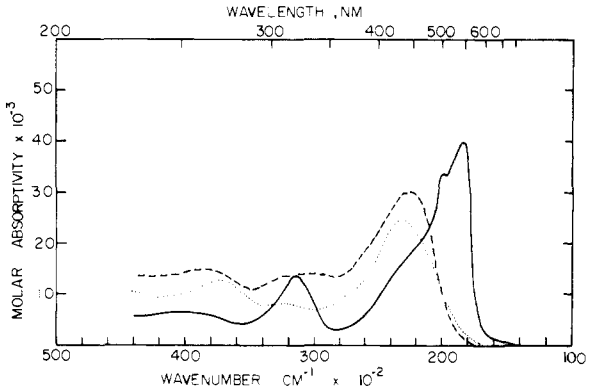


Figure 1. The room temperature (···), low temperature (- - -), and hydrogen halide cage perturbed (—) absorption spectra of the all-trans *n*-butyl retinal Schiff base + HCl.

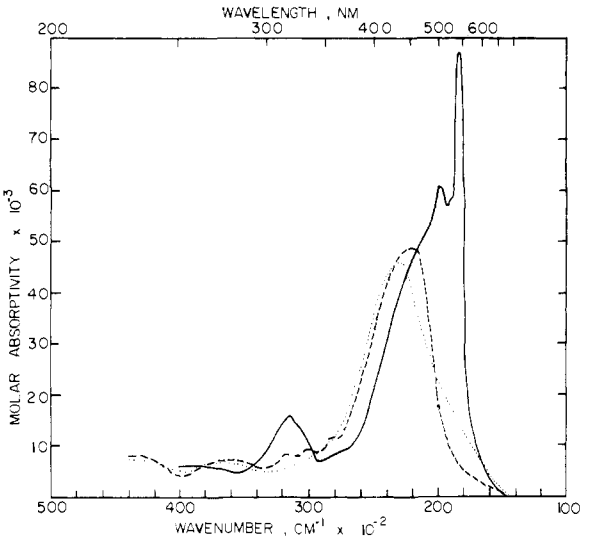


Figure 2. The room temperature (···), low temperature (- - -), and hydrogen halide cage perturbed (—) absorption spectra of the 11-cis *n*-butyl retinal Schiff base + HCl.

rescence excitation spectrum displays both of these bands (however, not the 460-nm band).

The room and low (77 K) temperature absorption spectra of the protonated all-trans methyl Schiff base are essentially identical with those for the *n*-butyl Schiff base, vide supra, including a long-wavelength 541-nm band and 318-nm peak. Recall that the absorption spectral properties of the nonprotonated methyl and *n*-butyl all-trans Schiff bases are also essentially identical.[32]

Protonation of the 11-cis *n*-butyl Schiff base in 3MP results in a shift in the room temperature absorption maximum from 350 to 438 nm. Cooling to 77 K results in a shift to 442 nm, with an increase in the molar absorptivity of ca. 20%. A second band can be observed in the 310–350-nm region and like the all-trans protonated Schiff base it has vibrational fine structure. At higher energies is a peak maximizing at 271 nm, Figure 2.

At 77 K, the long-wavelength absorbing species has band maxima at 543, 502, 318, and 270 nm. Figure 2 (the 523-nm shoulder is absent). When this solution is irradiated at 77 K the 318- and 270-nm bands decrease, while the 543-nm band intensity increases. Upon prolonged exposure to visible light at 77 K, the intensity of all bands is decreased.

Table II summarizes the absorption spectral properties of the all-trans and 11-cis *n*-butyl retinal Schiff base. Band maxima and oscillator strengths of the room temperature, low-temperature, and long wavelength absorbing species are given for all observed transitions above ca. 240 nm.

Addition of hydrogen bromide gas to the all-trans *n*-butyl Schiff base in 3MP and immediate cooling to 77 K resulted in a long-wavelength absorption band at 535 nm rather than 542 nm. However, owing to the insolubility of the HBr in 3MP and the insolubility of the protonated Schiff base at 77 K the solution was opaque, which made it impossible to obtain quantitative data on this complex.

Protonation of the all-trans Schiff base in a polar solvent, 2-methyltetrahydrofuran, with hydrogen chloride and cooling immediately to 77 K did not yield a long-wavelength absorption. The absorption spectrum maximized at 440 nm.

Discussion

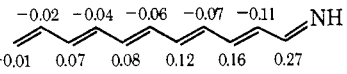
Since protonated retinal Schiff bases have first band maxima at ca. 440 nm,[15] the absorption spectrum recorded at 77 K having band maxima at 542 and 318 nm must represent a new definitive species. The experimental conditions optimal for the formation of this species are a nonpolar solvent, large excesses of anhydrous HCl gas, and low temperature. Three possible models (other than a conventionally protonated Schiff base) are a cationic species, a charge transfer species, or a protonated Schiff base in a HCl/solvent cage. As will be discussed below, the latter model best fits the experimental requirements. Theoretical calculations predict that a pure all-trans retinylic cation would absorb at ca. 600 nm.[37-39] However, the best evidence against a cationic species is that in 2-propanol/sulfuric acid at ca. −50 °C, it has an absorption at 603 nm.[40] A charge transfer model where the hydrogen chloride acts as an electron acceptor from the polyene π system is a possibility. However, if a charge transfer complex were to be formed, we would be unable to account for the dependence on the concentration of hydrogen chloride, and the temperature dependences at ca. 145 K.[41]

Table II. Experimental Band Maxima and Oscillator Strengths of Protonated Schiff Bases

	Transition I	Transition II	Transition III
	All-trans SB + HCl		
295 K	433 (1.47)	300-340 (0.09)	271 (0.18)
77 K	440 (1.33)	310-355 (0.14)	272 (0.17)
77 K	542 (1.83)	318 (0.26)	260-290 (0.12)
	11-*cis* SB + HCl		
295 K	438 (0.72)	310-340 (0.12)	268 (0.36)
77 K	442 (0.83)	310-340 (0.36)	271 (0.46)
77 K	543 (0.89)	318 (0.25)	260-270 (0.19)

Although the results of semiempirical molecular orbital calculations must be treated with caution, calculations combined with our experimental observations suggest a structure for this new species. Theoretical calculations show[42-45] that the position of the band maxima of protonated retinal Schiff bases is sensitive to negatively or positively charged groups in its environment, either at the nitrogen of the Schiff base[37,39,43,45] or at the opposite end of the polyene.[28,42,44] We have also observed this effect using the PPP method (Table I). However, as the results of Table I indicate, there is no unique way to account for this shift by calculations alone. For example, negative point charges above the chain, increased conjugation along the chain (i.e., decreasing the amount of single and double bond alteration), and increased delocalization of positive charge along the chain can all introduce a calculated red shift. Nevertheless, the formation of a solvent cage in which the polar, but nondissociated, hydrogen chloride (or hydrogen bromide) molecules can interact electrostatically with the polyene chain of the protonated Schiff base best fits the experimental requirements for the formation of the long-wavelength absorbing species. This interaction would result in a

stabilization of the polyene, and is supported by theoretical calculations which indicate that the positioning of a negative charge along the polyene chain can induce a red shift in the absorption maximum. CNDO calculations of the charge densities of a model protonated all-trans Schiff base indicate that the carbon atoms are alternately electropositive and electronegative.



Therefore, the δ^- chlorine atom of HCl would interact with the δ^+ carbon atoms of the polyene. Mathies and Stryer[46] have recently determined that all-trans retinal, its *n*-butylamine Schiff base, and its protonated Schiff base have a highly dipolar excited singlet state, thus indicating that Coulombic interactions with charged groups would result in a lowering of the transition energy of the chromophore by stabilization of its excited state. In our case, a solvent cage containing polar hydrogen chloride molecules could interact with the polyene carbon atoms preferentially stabilizing its excited singlet state (relative to the ground state).

Let us discuss our experimental results in light of the following three theories that have been used to explain the bathochromic shift between the absorption maxima of visual pigments and protonated Schiff bases: secondary protein–chromophore interactions of a Coulombic nature; interactions between the polarizable aromatic amino acids in opsin and the chromophore; and the proximity of the counteranion to the protonated Schiff base linkage. This latter theory is supported by theoretical calculations[22,37-39,42,43,45] and environmental studies of model protonated Schiff bases.[20-25,47,48] In our experiments the hydrogen chloride protonating species is not (essentially) dissociated and large excesses are required for the formation of the species with a long-wavelength absorption band at low temperatures. This indicates that factors in addition to the degree of protonation of the Schiff base linkage can induce large bathochromic shifts for protonated retinal Schiff bases.

Leermakers and co-workers[25-27] suggest that interactions between the chromophore and polarizable groups within the protein can lead to a lowering of the transition energy of the chromophore. Aromatic amino acids are present near the binding site of the chromophore in rhodopsin;[49,50] however, theoretical calculations[42,43] indicate that the polarizability of the chromophore environment in the protein would not induce bathochromic shifts of the mangitude found in visual pigments.

Hubbard and Kropf[18,19] suggest that the band maxima of visual pigments is at lower energies as a result of secondary noncovalent interactions between opsin and the polyene. The data reported here support this theory and the slightly modified theory of Honig and Ebrey.[28] In our case the interactions occur between the chlorine atom of the polar hydrogen chloride molecules that help comprise the solvent cage and the carbon atoms of the π electronic system of the protonated Schiff base.[51]

One result that may have important implications in visual pigments is that experimentally, we have demonstrated that sufficient stabilization is imparted by the presence of one or more electronegative atoms and that an anion need not be present, which agrees with calculations of Honig, Ebrey, and co-workers.[28]

Our results also indicate that in our low-temperature solvent cages, the geometry of the chromophore is unimportant in determining the transition energy of the protonated Schiff base since the shape of the equilibrium solvent cage would presumably alter to optimize the Coulombic interactions with the polyene. In visual pigments the magnitude of the electrostatic interactions would be controlled largely by the distance of the polyene to the stabilizing species since the mobility of these groups is expected to be largely inhibited. Previous theoretical calculations[21,42,44] and the calculations presented here indicate that the transition energy is dependent upon the position and magnitude of the negative charge in relationship to the polyene.

There is evidence that the protein in rhodopsin undergoes no significant conformational changes until after lumirhodopsin intermediate.[52,53] If this is the case then the decay of lumirhodopsin to metarhodopsin I represents a weakening in the protein–chromophore interaction as a result of the unfolding of the protein. The small energy differences (ca. 450 cm^{-1}) between the absorption maxima of rhodopsin, isorhodopsin, 9,13-isorhodopsin (isorhodopsin II), and lumirhodopsin probably result from small differences in distance between the chromophore in these pigments and one particular site of secondary interaction. The bathorhodopsin (prelumirhodopsin) bleaching intermediate is unique since its long-wavelength adsorption maximum is ca. 40–50 nm to the red of all other pigments and cannot be explained on the basis of secondary Coulombic interactions alone. As previously suggested by Yoshizawa and Wald,[52] the bathorhodopsin intermediate may be highly twisted about double bonds, although an alternative structure has been proposed.[54]

Several comments about the structural features of protonated Schiff bases (λ_{max} 440 nm) should be made. As was the case in the isomeric retinals[55-57] and their Schiff bases,[32,36] the second observed band in protonated Schiff bases shows fine structure in both the all-trans (HCl, HBr) and 11-cis (HCl) isomers. We assign the second observed band as a $\pi^* \leftarrow \pi$ transition in the protonated retinal Schiff bases.

The 11-cis protonated Schiff base (λ_{max} ca. 438 nm) has several interesting spectral differences compared to the trans form. The 271-nm band of 11-cis retinal Schiff base (λ_{max} 438 nm) is very intense and is assigned as the "cis" band[15] ("A_g^-" using C_{2h} symmetry notation) arising from a higher $\pi^* \leftarrow \pi$ transition. Upon cooling a room temperature solution of the 11-cis protonated Schiff base, there is a small red shift in the first band to 442 nm and increase in the oscillator strength by 15–20%. This spectral behavior is similar to that of 11-cis retinal[56-59] and its *n*-butyl Schiff base,[32] and has been interpreted as resulting from a redistribution of the populations of the 12-s-cis and 12-s-trans conformations with temperature,[32,60-63] with the 12-s-trans predominating at lower temperatures.[32,59]

Summary

The protonated Schiff base of 11-cis retinal has absorption spectral properties that parallel those of retinals and nonprotonated Schiff bases including a temperature-sensitive first band maxima, vibrational structure in the second observed $\pi^* \leftarrow \pi$ transition, and a "cis" peak at higher energies.

Cooling a solution of the all-trans or 11-cis retinal Schiff base to 77 K immediately after addition of anhydrous hydrogen chloride gas results in the formation of a species with a long-wavelength absorption maximum at ca. 540 nm with related bands.[64] With the aid of theoretical calculations, we conclude that this low-energy transition of the polyene is the result of a preferential stabilization (relative to the ground state) of the excited singlet state caused by the formation of a solvent cage containing the polar hydrogen chloride molecules where the chlorine atom can interact with the δ^+ atoms of the polyene. The electrostatic nature of these interactions gives experimental evidence that is consistent with the suggestion by Hubbard and Kropf[18,19] and that of Honig and Ebrey[28] that in rhodopsin the transition of the chromophore is at a lower energy as a result of interactions with the protein environment. It is thus possible to explain the low-energy transition of rho-

dopsin, its bleaching intermediates and synthetic pigments using the Hubbard and Kropf model.

Acknowledgments. One of the authors (W.H.W.) wishes to thank Dr. R. R. Birge for providing a manuscript of his work prior to publication, and Dr. B. Honig for helpful discussions. We are indebted to the National Eye Institute, National Institutes of Health, Grant EY 00875, for their generous support of this work.

References and Notes

(1) Presented at the 165th National Meeting of the American Chemical Society, Dallas, Texas, April 1973. Part 6: R. S. Becker, G. Hug, P. K. Das, A. M. Schaffer, T. Takemura, N. Yamamoto, and W. H. Waddell, *J. Phys. Chem.*, **80**, 2265 (1976).
(2) To whom correspondence should be addressed.
(3) R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36**, 269 (1952); G. Wald, *Science*, **162**, 230 (1968), and references cited therein.
(4) F. D. Collins, *Nature (London)*, **171**, 469 (1953).
(5) R. A. Morton and G. A. J. Pitt, *Biochem. J.*, **59**, 128 (1955).
(6) A. R. Oseroff and R. H. Callender, *Biochemistry*, **13**, 4243 (1974).
(7) A. Lewis, R. S. Fager, and E. W. Abrahamson, *J. Raman Spectrosc.*, **1**, 465 (1973).
(8) R. Mathies, A. R. Oseroff, and L. Stryer, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1 (1976).
(9) R. H. Callender, A. Doukas, R. Crouch, and K. Nakanishi, *Biochemistry*, **15**, 1621 (1976).
(10) D. Bownds, *Nature (London)*, **216**, 1178 (1967).
(11) M. Akhtar, P. T. Blosse, and P. B. Dewhurst, *Biochem. J.*, **110**, 693 (1968).
(12) W. J. DeGrip, S. L. Bonting, and F. J. M. Daemen, *Biochim. Biophys. Acta*, **303**, 189 (1973).
(13) R. S. Fager, P. Sejnowski, and E. W. Abrahamson, *Biochem. Biophys. Res. Commun.*, **47**, 1244 (1973).
(14) G. Wald and P. K. Brown, *J. Gen. Physiol.*, **37**, 189 (1953).
(15) For a review see B. Honig and T. G. Ebrey, *Annu. Rev. Biophys. Bioeng.*, **3**, 151 (1974).
(16) E. W. Abrahamson and S. E. Ostroy, *Prog. Biophys. Mol. Biol.*, **17**, 179 (1967).
(17) R. Crouch, V. Purvin, K. Nakanishi, and T. G. Ebrey, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 1538 (1975).
(18) R. Hubbard, "Visual Problems of Color", W. S. Stiles, Ed., H. M. Stationery Office, 1956, pp 151–169.
(19) A. Kropf and R. Hubbard, *Ann. N.Y. Acad. Sci.*, **74**, 266 (1958).
(20) J. O. Erickson and P. E. Blatz, *Vision Res.*, **8**, 1367 (1968).
(21) P. E. Blatz and J. H. Mohler, *Chem. Commun.*, 614 (1970).
(22) P. E. Blatz, *Photochem. Photobiol.*, **15**, 1 (1972).
(23) P. E. Blatz, J. H. Mohler, and H. V. Navangul, *Biochemistry*, **11**, 848 (1972).
(24) P. E. Blatz and J. H. Mohler, *Biochemistry*, **11**, 3240 (1972).
(25) C. S. Irving and P. A. Leermakers, *Photochem. Photobiol.*, **7**, 665 (1968).
(26) C. S. Irving, G. W. Byers, and P. A. Leermakers, *J. Am. Chem. Soc.*, **91**, 2141 (1969).
(27) C. S. Irving, G. W. Byers, and P. A. Leermakers, *Biochemistry*, **9**, 858 (1970).
(28) B. Honig, A. D. Greenberg, U. Kinur, and T. G. Ebrey, *Biochemistry* **15**, 4593 (1976).
(29) W. H. Waddell and R. S. Becker, *J. Am. Chem. Soc.*, **93**, 3788 (1971).
(30) A. Streitwelser, Jr., "Molecular Orbital Theory", Wiley, New York, N.Y., 1961.
(31) A. M. Schaffer, M. Gouterman, and E. R. Davidson, *Theor. Chim. Acta*, **30**, 9 (1973).
(32) A. M. Schaffer, W. H. Waddell, and R. S. Becker, *J. Am. Chem. Soc.*, **96**, 2063 (1974).
(33) S. Mataga and N. Mataga, *Z. Phys. Chem. (Frankfurt am Main)*, **19**, 231 (1959).
(34) K. Nishimoto, K. Nakatsukasa, and R. Fujishiro, *Theor. Chim. Acta*, **14**, 80 (1969).
(35) Program 141, Quantum Chemistry Program Exchange.
(36) W. H. Waddell, A. M. Schaffer, and R. S. Becker, *J. Am. Chem. Soc.*, **95**, 8223 (1973).
(37) H. Suzuki, N. Takizawa, and T. Komatsu, *Suppl. Prog. Theor. Phys.*, **46**, 16 (1970).
(38) H. Suzuki, T. Komatsu, and T. Kato, *J. Phys. Soc. Jpn.*, **34**, 156 (1973).
(39) H. Suzuki, T. Komatsu, and H. Kitajima, *J. Phys. Soc. Jpn.*, **37**, 177 (1974).
(40) P. E. Blatz and D. L. Pippert, *Chem. Commun.*, 176 (1968).
(41) A charge transfer complex may be formed upon addition of HCl gas to all-trans retinal in 3MP. At room temperature, there is a small red shift from 369 to 371 nm, but upon cooling to ca. −30 °C a peak at ca. 650 nm can be observed. At 77 K this peak dominates the absorption spectrum. These spectra are completely reversible with temperature between room temperature and 77 K, unlike the 542-nm band of the protonated Schiff bases.
(42) For a review see B. Honig, A. Warshel, and M. Karplus, *Acc. Chem. Res.*, **8**, 92 (1975).
(43) M. J. Mantione and B. Pullman, *Int. J. Quantum Chem.*, **5**, 349 (1971).
(44) A. Waleh and L. I. Ingraham, *Arch. Biochem. Biophys.*, **156**, 261 (1973).
(45) J. R. Wiesenfeld and E. W. Abrahamson, *Photochem. Photobiol.*, **8**, 487 (1968); E. W. Abrahamson and J. R. Wiesenfeld in "Biochemistry and Physiology of Visual Pigments", H. Langer, Ed., Springer-Verlag, New York, N.Y., 1973.
(46) R. Mathies and L. Stryer, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2169 (1976).
(47) Y. Koto, T. Suzuki, M. Azuma, and Y. Sekoguti, *Nature (London)*, **218**, 955 (1968).
(48) T. Suzuki and Y. Kito, *Photochem. Photobiol.*, **15**, 275 (1972).
(49) K. Shirane, *Nature (London)*, **254**, 722 (1975).
(50) A. Cooper and M. E. Hogan, *Biochem. Biophys. Res. Commun.*, **68**, 178 (1976).
(51) The explanation of the 542-nm band (ref 29) has been reinterpreted based upon recent information derived from theoretical calculations and the data presented here.
(52) T. Yoshizawa and G. Wald, *Nature (London)*, **197**, 1279 (1963).
(53) T. G. Ebrey and T. Yoshizawa, *Exp. Eye Res.*, **17**, 545 (1973).
(54) M. R. Fransen, W. C. M. Luyten, J. van Thuijl, J. Lugtenburg, P. A. A. Jensen, P. J. van Breugel, and F. J. M. Daemen, *Nature (London)*, **260**, 726 (1976).
(55) W. S. Sperling and C. N. Rafferty, *Nature (London)*, **224**, 591 (1969).
(56) R. S. Becker, K. Inuzuka, and D. E. Balke, *J. Am. Chem. Soc.*, **93**, 38 (1971).
(57) R. S. Becker, K. Inuzuka, J. King, and D. E. Balke, *J. Am. Chem. Soc.*, **93**, 43 (1971).
(58) L. Jurkowitz, J. N. Loeb, P. K. Brown, and G. Wald, *Nature (London)*, **184**, 614 (1959).
(59) R. Rowan, A. Warshel, B. D. Sykes, and M. Karplus, *Biochemistry*, **13**, 970 (1974).
(60) B. Honig and M. Karplus, *Nature (London)*, **229**, 558 (1971).
(61) A. Warshel and M. Karplus, *J. Am. Chem. Soc.*, **96**, 5677 (1974).
(62) R. R. Birge, K. Schulten, and M. Karplus, *Chem. Phys. Lett.*, **31**, 451 (1975).
(63) R. R. Birge, M. J. Sullivan, and B. E. Kohler, *J. Am. Chem. Soc.*, **98**, 358 (1976).
(64) The absorption spectrum of the protonated all-trans retinal Schiff base with band maximum at 542 nm has been reproduced in order to make Raman measurements. M. Birth, K. Schulten and M. Stockburger, The Bathochromic Shift in Protonated Schiff's Bases Amines-Resonance Raman Study, private communication.