

Wavelength regulation in rhodopsin: Effects of dipoles and amino acid side chains

(point charge/perturbation)

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ABSTRACT The effects of dipoles and aromatic amino acid side-chain models on the absorption and optical activity of the rhodopsin chromophore were calculated by using perturbation theory, and the results were compared with those of a Pariser-Parr-Pople calculation for the unperturbed system. The interaction was assumed to result from purely electrostatic interactions. It was concluded that the side chains of phenylalanine and tryptophan should have no important effects. However, the charge separation in tyrosine is sufficient to cause substantial electrostatic perturbation; in fact, the effect of tyrosine is large enough to approximate many of the spectral properties of rhodopsin quantitatively. This is encouraging because the use of aromatic amino acid side-chain analogs probably provides a better physical model than the use of isolated full charges, except in the case of the counterion to the protonated Schiff base.

The effect of the interaction between the protein opsin and 11-*cis*-retinal has been a matter of considerable interest, both experimentally and theoretically. Two of the most important results of this interaction are a marked red shift in the absorbance maximum from that of the retinylidene Schiff base and the appearance of a marked optical activity. Both effects have been the subject of a number of theoretical investigations.

A major cause of the red shift of the rhodopsins is the protonation of the Schiff base. This was implied by the observed effect of protonation on spectra of model compounds (1, 2) and has received further support from experimental (3, 4) and theoretical (5-7) studies. However, protonation by itself is insufficient; the resulting red shifts of model compounds are less than those observed in most rhodopsins and protonation by itself cannot account for the wide range of wavelength maxima observed in rhodopsins from different species (8).

Additional causes that have been considered include separate charges near the chromophore (5, 6, 9, 10), bond twisting (5, 7), and polarizability of the protein matrix (11). All three should be capable of causing quite specific perturbation effects that could be sensitive to the conformation of the protein and thus potentially fulfill the function of specific wavelength control. In this paper, we present a variation on the perturbing-charge model—i.e., perturbing dipoles. Although the basic idea is not new (6, 8), it has not received the attention that it might. In fact, this model is perhaps more satisfactory from a physical standpoint, if one considers the classical oil-drop model of protein organization, because it does not require the introduction of charges that lack counterions into the hydrophobic region of the protein.

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DESCRIPTION OF THE MODEL

The zero-order system used is shown in Fig. 1. The parameters derived by using a Pariser-Parr-Pople calculation were provided to us (B. Honig, personal communication), and the effect of the additional dipoles was calculated by using perturbation theory (12). In this case, the perturbation was calculated as resulting from the interaction between the electric field due to the point-charge distribution of the perturbant and the π system:

$$\hat{\mathcal{H}}' = \sum_i V_i = \sum_i \sum_j -Z_i e^2 / r_{ij}, \quad [1]$$

where i is the i th point charge, Z_i is its charge, j is the j th electron, and r_{ij} is the distance between the i th charge and the j th electron. Because r_{ij} involves only the j th electron, $\hat{\mathcal{H}}'$ is a one-electron operator. This reduces the expression for the perturbation interaction between states $\psi_{p \rightarrow q}$ and $\psi_{s \rightarrow t}$ to

$$\hat{\mathcal{H}}'_{pqst} = \langle \phi_q | \hat{\mathcal{H}}' | \phi_q \rangle - \langle \phi_p | \hat{\mathcal{H}}' | \phi_p \rangle \quad [2]$$

and

$$\hat{\mathcal{H}}'_{pqst} = \langle \phi_q | \hat{\mathcal{H}}' | \phi_t \rangle \delta_{ps} + \langle \phi_p | \hat{\mathcal{H}}' | \phi_s \rangle \delta_{qt} \quad [3]$$

for the diagonal and off-diagonal terms, respectively, where $\psi_{p \rightarrow q}$ represents the excited singlet state for the transition $p \rightarrow q$, ϕ_q is the molecular orbital expressed as a linear combination of atomic orbitals (LCAO-MO) of q , and δ_{ab} is the Kronecker delta function. In Eq. 2, the energy is expressed relative to the ground state energy.

Because the properties we are concerned with here are the electric- and magnetic-transition dipole moments, the operators are, respectively,

$$\hat{\mu} = \sum_j e \vec{r}_j \quad [4]$$

and

$$\hat{m} = -eh/4\pi mc \sum_j \vec{r}_j \times \vec{\nabla}_j \quad [5]$$

or, if $\hat{\mu}$ is in Debye units, \hat{m} is in Bohr magnetons (μ_B), and \vec{r} is in angstroms,

$$\hat{\mu} = 4.8 \sum_j \vec{r}_j \quad [6]$$

and

$$\hat{m} = -i \sum_j \vec{r}_j \times \vec{\nabla}_j. \quad [7]$$

Abbreviation: LCAO-MO, molecular orbital expressed as a linear combination of atomic orbitals.

For the transition from the ground state to the zero-order, configuration-interaction state a , the resulting transition dipoles are

$$\vec{\mu}_{oa}^0 = 4.8 \sqrt{2} \sum_p \sum_q A_{apq} \sum_{k=1}^n \sum_{l=1}^n C_{pk} C_{ql} \langle \chi_k | \vec{r} | \chi_l \rangle \quad [8]$$

and

$$\vec{m}_{oa}^0 = -i \sqrt{2} \sum_p \sum_q A_{apq} \sum_{k=1}^n \sum_{l=1}^n C_{pk} C_{ql} \langle \chi_k | \vec{r} \times \vec{\nabla} | \chi_l \rangle, \quad [9]$$

where k and l are atoms or their corresponding atomic orbitals, C_{pk} is the LCAO-MO coefficient for atomic orbital k in molecular orbital p , and A_{apq} is the configuration-interaction mixing coefficient for transition $p \rightarrow q$ in state a . These zero-order states are further mixed by the perturbation.

The electric- and magnetic-transition dipole moments are not directly observable. Rather, they are related to the experimentally determinable oscillator strength (f_{oa}) and rotational strength (R_{oa}) by

$$\begin{aligned} f_{oa} &= (4.7/\lambda) |\vec{\mu}_{oa}|^2 \\ R_{oa} &= I_m(\vec{\mu}_{oa} \cdot \vec{m}_{oa}) \end{aligned} \quad [10]$$

where λ is in nanometers, μ is in Debye units, and m is in Bohr magnetons.

Two types of perturbants were used, pure dipoles and aromatic amino acid side-chain analogs. In the first case, the dipoles consisted of two equal but oppositely charged point charges having arbitrary separation and orientation with respect to the conjugated system. For the second case, effective atomic charges and interatomic distances for the side chains were taken from the literature (13). To simplify the calculations, the charges on hydrogens bound to carbons were included with those of the carbons as one atom at the carbon atom coordinates. In addition, all carbon atoms not bound to elements other than carbon or hydrogen were ignored as having insignificant effective atomic charges.

One problem caused by the use of this perturbation system was that the effect of the perturbing charges on the electronic and geometric structure of the chromophore could not be included directly. To estimate the effect of accommodation by the chromophore, calculations were performed that were analogous to altering the distance between the Schiff base nitrogen and the counterion A_1^- (Fig. 1). Comparison of these calculations with the results of a Pariser-Parr-Pople calculation on a slightly different geometry (Fig. 2) showed that an effective dielectric constant (ϵ) of 2 gives a better fit than does a value of 1; thus, all of the calculations involving model amino acid side chains used effective charges only one-half the magnitude of the literature values.

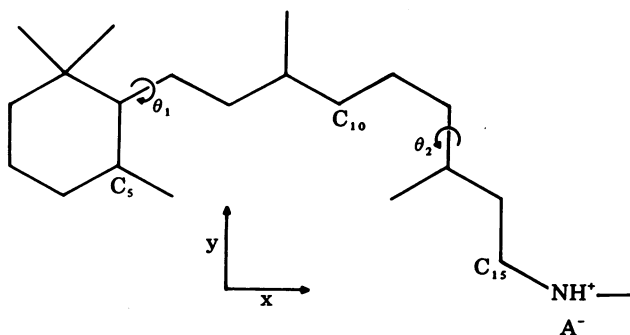


FIG. 1. Geometry of the system. $\theta_1 = -140^\circ$, $\theta_2 = 40^\circ$; coordinates (x, y, z) for C_5 , C_{10} , and C_{15} are $(-5.65, -1.59, 0.75)$, $(-0.63, -0.37, 0)$, and $(2.70, -3.92, -0.75)$, respectively.

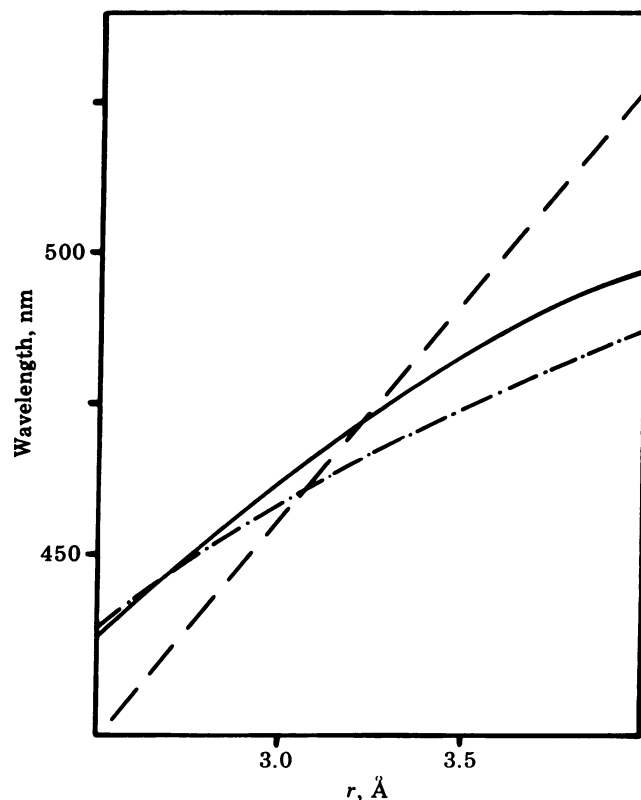


FIG. 2. Effect of distance between nitrogen and counterion. —, All-trans form (ref. 6); ---, $\epsilon = 1$; - · - ·, $\epsilon = 2$.

Effect of Dipole. Perturbation by a dipole should be both position and orientation dependent. Both theoretical calculations (5, 10, 14) and experimental results (15) suggest that rhodopsin has a large electric-transition dipole moment, in which the electron density shifts from the ring end of the molecule to the Schiff base end. As a result, an anion placed near the ring end should cause a red shift in the absorption spectrum and one placed near the Schiff base should cause a blue shift. The reverse is true for a cation. Similarly, a dipole that has its negative end nearer the ring should cause a red shift in the absorption spectrum, one that has its positive end nearer should cause a blue shift, and one in which the two ends are equidistant should have little if any effect.

These expectations were borne out by comparison of results for unperturbed conditions and the experimental results (Table 1) and the results of the perturbation calculations (Table 2). The hypothetical dipole used had charges of ± 1 separated by a distance of 1 Å, and the x , y , and z coordinates were taken as for the center of the bond. The rotation occurred in the xy plane and was defined as follows: for $\theta = 0^\circ$, the dipole is parallel to the region of the conjugated chain closest to it and has its negative end closer to the ring; for $\theta = 90^\circ$, the dipole is perpendicular to the molecular axis and has its positive end turned

Table 1. Results for comparison of positional and rotational dependence of perturbation

	λ_1 , nm	μ_1 , D	R_1 , D $\cdot\mu_B$	λ_2 , nm	μ_2 , D	R_2 , D $\cdot\mu_B$
Unperturbed conditions	456	6.76	0.617	308	4.19	-0.629
Experimental results	498*	8.91†	0.53*	345*	—	—

* See ref. 16.

† See ref. 17.

Table 2. Positional and rotational dependence of perturbation

$x, \text{\AA}$	$y, \text{\AA}$	$z, \text{\AA}$	$\theta, ^\circ$	λ_1, nm	μ_1, D	$R_1, \text{D}\cdot\mu_B$	λ_2, nm	μ_2, D	$R_2, \text{D}\cdot\mu_B$
By dipole									
-3.06	-0.42	3.00	0	518	6.86	0.460	329	3.11	-0.527
			90	458	6.67	0.650	308	4.08	-0.844
			180	422	6.56	0.817	304	5.01	0.147
			270	455	6.84	0.584	308	4.27	-0.487
0.63	0.36	3.00	0	511	6.73	0.528	320	3.40	-0.738
			90	470	6.68	0.573	310	3.54	-0.822
			180	434	6.58	0.712	303	5.03	0.052
			270	443	6.84	0.666	307	4.69	-0.344
4.00	0.10	-1.90	0	522	6.77	0.595	318	4.18	-0.626
			90	468	6.72	0.622	310	4.25	-0.551
			180	410	6.73	0.646	306	4.80	-0.242
			270	466	6.79	0.618	314	4.53	-0.458
By tryptophan									
-3.0	0	3.0	0	452	6.73	0.642	307	4.44	-0.541
			90	459	6.75	0.610	307	4.46	-0.535
			180	466	6.77	0.595	309	4.02	-0.728
			270	455	6.72	0.624	306	4.46	-0.547
0	0	3.0	0	448	6.74	0.644	306	4.30	-0.538
			90	455	6.73	0.618	307	4.24	-0.609
			180	463	6.75	0.610	310	4.09	-0.670
			270	452	6.75	0.627	307	4.42	-0.511
3.5	-4.5	1.5	30	444	6.83	0.643	305	4.56	-0.428
			120	457	6.73	0.605	308	4.13	-0.647
			210	464	6.70	0.593	310	3.83	-0.755
			300	450	6.78	0.634	306	4.44	-0.505
By tyrosine									
-4.23	0.26	3.00	90*	516	6.81	0.548	332	3.43	-0.623
			180*	518	6.82	0.541	332	3.41	-0.612
			270*	518	6.82	0.544	332	3.42	-0.613
			90†	507	6.83	0.532	329	3.27	-0.625
0.64	0.36	3.00	90*	469	6.66	0.778	322	3.99	-1.332
			180*	472	6.66	0.884	333	4.52	-1.027
			270*	468	6.66	0.779	322	4.02	-1.306
			90†	485	6.69	0.682	322	3.82	-1.137
5.49	-4.56	0.83	90*	420	6.93	0.762	307	4.69	-0.184
			170*	419	6.93	0.767	307	4.69	-0.178
			270*	419	6.93	0.769	307	4.69	-0.176
			90†	422	6.87	0.756	308	4.72	-0.169

* $\phi = 55^\circ$.† $\phi = 0^\circ$.

toward the negative y direction; for $\theta = 180^\circ$ and $\theta = 270^\circ$, the results may be easily determined from the first two angles. As expected, for $\theta = 0^\circ$, the λ_1 values show considerable red shifts; for $\theta = 180^\circ$, the λ_1 values show blue shifts of approximately the same magnitude; and for $\theta = 90^\circ$ and $\theta = 270^\circ$, the λ_1 values show generally small shifts. For λ_2 , the trends are the same, although the shifts are much less. The greater sensitivity of λ_1 is due to the larger shift in electron density for this transition, because the first-order perturbation energy of ψ_n depends only on ψ_n^0 and \mathcal{H}' (12). In the case of the electric-transition dipole moments and rotational strengths, the relative sensitivities are reversed. This is because the first transition ($E_1^0 = 2.72$ eV) is energetically quite separate from the others, but the next three transitions ($E_2^0 = 4.03$ eV, $E_3^0 = 4.17$ eV, and $E_4^0 = 4.83$ eV) are close together and therefore mix much more. The wavelength range for λ_1 in these few results (410–522 nm) and the sensitivity of other properties would seem to indicate that dipole perturbation should contribute measurably to the rhodopsin spectrum if any such dipoles are present near the chromophore.

Model Amino Acids. To make our results more meaningful in terms of how the protein could cause specific effects on the chromophore, we investigated the effects of aromatic amino

acid side chains. The parameters used were modified from literature values by using the simplification described above (Table 3).

For the case of tryptophan, the only portion of the molecule used was the heterocyclic ring. For this series, the angle θ is equivalent to the θ given above, except that the orientation vector is from the middle of the ring to the nitrogen—e.g., when this vector is parallel to the molecular axis of the chromophore

Table 3. Molecular parameters of tryptophan and tyrosine*

Amino acid	Atom	$x, \text{\AA}$	$y, \text{\AA}$	Charge, e
Tryptophan	N	0.00	-0.85	-0.269
	H	0.00	-1.80	0.091
	C_α	1.40	-0.37	0.078
	C'_α	-1.40	-0.37	0.078
	C_β	0.70	0.84	0.018
Tyrosine	C'_β	-0.70	0.84	0.018
	C	0.00	1.36	0.140
	O	0.00	0.00	-0.471
	H	0.94	-0.34	0.209

* Adapted from ref. 13.

and the nitrogen is toward the Schiff base end, $\theta = 0^\circ$. Because the nitrogen is the negative portion of the ring, it is expected that the effects produced here would be offset by 180° from those of the dipole.

Comparison of the data in Table 2 generally bears out the expectations from the results of the dipole calculations. Wavelength shifts, dipole moment changes, and the rotational strength of both transitions are all generally 180° out of phase. The most noticeable difference, however, is the magnitude of the perturbation by tryptophan. The distribution of positive charges on both sides of the negative nitrogen results in point-charge perturbations that nearly cancel each other. It is really no surprise, then, that the effect should be so weak. This does not agree with the experimental results of Kliger *et al.* (18) for the protonated Schiff base of retinal, which show a marked effect for the indole ring. However, Kliger *et al.* assumed the major cause of tryptophan perturbation to be polarizability, which, for two reasons, is a more likely possibility. First, the effect calculated for the heterocyclic ring is small because of effects that cancel one another and because the six-carbon ring is ignored due to its lack of significant dipoles; thus, the indole ring will not have a large perturbing effect due to dipolar interaction, even under a favorable orientation. Second, because the heterocyclic ring is between the peptide backbone and the benzene-like ring, its ability to come in close contact with the chromophore will be physically limited. The dipolar portion of the tryptophan effect is thus relatively unimportant except possibly for electronic states of nearly degenerate energies.

For the case of tyrosine, the net charges on all of the CH pairs are so small that they were ignored and only the C—OH bond was considered. A slight variation was made in the definition of orientation angles: θ is the angle between the O—C bond and the major vector of the chromophore (x axis for carbons 6–13), and ϕ is the angle between the O—C bond and the vector perpendicular to the plane of the nearby molecular region. For the case when $\phi = 55^\circ$, the oxygen atom is closest to the chain and the carbon and hydrogen are further above.

Unlike the results for the dipole and for tryptophane, the results for tyrosine show almost no orientational effects. This is especially true when the carbon and hydrogen point away from the molecular chain, but even when the hydrogen is allowed to approach to a distance similar to that of the oxygen, the results are hardly changed. Thus, as long as the oxygen is at least as close to the chromophore as the other two atoms, its effect will be dominant because of its greater charge. One should not conclude that the system can be treated as a single point charge, however, because it was not possible to reproduce these results by using a single charge (calculations not shown).

Some agreement was found between these results and those of a calculation in which a small point-charge anion was used, but they were only qualitative. Positioning the group near the ring end caused a pronounced red shift, increased the electric-transition dipole, and decreased the rotational strength of the first peak, as is the case for a small anion in that region. Relative to the wavelength shift, however, tyrosine had a much greater effect on both the electric-transition dipole moment and the rotational strength, the effects on the three being equivalent to charges of -0.25 , -0.7 , and -0.9 , respectively, compared with the partial charge on the oxygen of -0.471 . Placed near the Schiff base, the phenol moiety again showed qualitative, but not quantitative, agreement with an isolated point charge.

As far as the implications for rhodopsin, Table 2 shows one very promising and undoubtedly fortuitous set of data, the results for the tyrosine dipole near the ring end. Comparison of

these results, those of the unperturbed system, and the experimental values shows that the tyrosine-perturbed system is much closer to the experimental. The values for λ_1 , R_1 , and λ_2 are all much closer to the perturbed case; only μ_1 fails to show much of an improvement. Lacking quantitative results for μ_2 and R_2 , it is nonetheless possible to make order-of-magnitude estimates and sign comparisons. Because the absorbance of peak two is so low, the calculated value of 4.19 D for the unperturbed state is probably not as good as the 3.38 D average found for the phenol moiety near the ring end. Finally, there is practically no effect of tyrosine on R_x , even though this is the worst of the six values, being very negative although the experimental value is very positive. The large effects shown in these calculations thus complement the experimental work (18).

Taken together, these results suggest that the tyrosine moiety is potentially an important side chain for dipolar interactions for three main reasons: (i) Because it is quite nonpolar, it would fit well into the highly hydrophobic region occupied by the rhodopsin (19) without presenting the unfavorable energetics of an ion (20); (ii) its dipole is of sufficient magnitude to produce marked shifts in spectroscopic quantities; and (iii) because its dipolar region is at the end of the phenyl ring away from the peptide backbone, it would not face the steric restrictions that, for example, tryptophan might.

One major limitation of this work is that the calculations did not show an appreciable effect by tyrosine in the region of carbon 12. This region has been shown to be important in wavelength regulation (21), so this failure should cast doubts on the quantitative nature of the results. However, the qualitative conclusions that tyrosine should be capable of a major role in wavelength regulation via electrostatic effects and that a single point charge does not adequately represent the effects of a polarized polyatomic species should still be valid.

CONCLUSION

Considerable problems remain for theoretical and experimental chemists hoping to elucidate the structure of the rhodopsin chromophore and its protein environment. Not only does a model need to explain the observed spectra, it must also account for the kinetics and spectra of the intermediates in the bleaching process. This work has shown that dipole perturbation of the chromophore could be an important factor in regulating the optical properties of rhodopsin and that more exact calculations, especially for tyrosine, should be made. Such results would complement the calculation for the nonspecific effects of polarizability and of small permanent dipoles in the general protein matrix (11).

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1. Morton, R. A. & Pitt, G. A. J. (1955) *Biochem. J.* **59**, 128–134.
2. Erickson, J. O. & Blatz, P. E. (1968) *Vision Res.* **8**, 1367–1375.
3. Callender, R. H., Doukas, A., Crouch, R. & Nakanishi, K. (1976) *Biochemistry* **15**, 1621–1629.
4. Oseroff, A. R. & Callender, R. H. (1974) *Biochemistry* **13**, 4243–4248.
5. Weisenfeld, J. & Abrahamson, E. W. (1968) *Photochem. Photobiol.* **8**, 487–493.
6. Honig, B., Greenberg, A. D., Dinur, U. & Ebrey, T. G. (1976) *Biochemistry* **15**, 4593–4599.
7. Suzuki, H., Komatsu, T. & Kitajima, H. (1974) *J. Phys. Soc. Jpn.* **37**, 177–185.
8. Dartnall, H. J. A. & Lythgoe, J. N. (1965) *Vision Res.* **5**, 81–100.
9. Kropf, A. & Hubbard, R. (1958) *Ann. N.Y. Acad. Sci.* **74**, 266–280.

10. Waleh, A. & Ingraham, L. L. (1973) *Arch. Biochem. Biophys.* **156**, 261–266.
11. Warshel, A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2558–2562.
12. Eyring, H., Walter, J. & Kimball, G. E. (1944) *Quantum Chemistry* (Wiley, New York).
13. Del Re, G., Pullman, B. & Yonezawa, T. (1963) *Biochim. Biophys. Acta* **75**, 153–182.
14. Waddell, W. H., Schaffer, A. M. & Becker, R. S. (1977) *J. Am. Chem. Soc.* **99**, 8456–8460.
15. Mathies, R. & Stryer, L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2169–2173.
16. Waggoner, A. S. & Stryer, L. (1971) *Biochemistry* **10**, 3250–3254.
17. Shichi, H., Lewis, M. S., Irreverre, F. & Stone, A. L. (1969) *J. Biol. Chem.* **244**, 529–536.
18. Kliger, D. S., Milder, S. H. & Dratz, E. A. (1977) *Photochem. Photobiol.* **25**, 277–286.
19. Hirtenstein, M. D. & Akhtar, M. (1970) *Biochem. J.* **119**, 359–366.
20. MacDonald, R. C. (1976) *Biochim. Biophys. Acta* **448**, 193–198.
21. Honig, B., Dinur, U., Nakanishi, K., Balogh, Nair, V., Gawinowicz, M. A., Arnaboldi, M. & Motto, M. G. (1979) *J. Am. Chem. Soc.* **101**, 7084–7086.