THEORETICAL STUDIES OF THE ELECTROCHROMIC RESPONSE OF CAROTENOIDS IN PHOTOSYNTHETIC MEMBRANES

TOSHIAKI KAKITANI, BARRY HONIG, AND ANTONY R. CROFTS
Department of Physiology and Biophysics, The University of Illinois, Urbana, Illinois 61801

ABSTRACT Molecular orbital calculations are carried out on a number of carotenoids in the presence of an external charge and a constant electric field. The external charge is used to represent the strong permanent field that is believed to polarize carotenoids in photosynthetic membranes and thus to account for their linear response to the transmembrane potential. Our calculations show that the in vitro → in vivo spectral shifts of carotenoids (~25 nm) can be produced by a charge in close proximity to the molecule. The interaction of the induced dipole moment with a constant field accounts for the observed magnitude of the electrochromic response in photosynthetic bacteria. The existence of a second pool of carotenoids that shows a significant (~20 nm) wavelength shift but no electrochromic response can be explained by an external charge positioned near the center of the molecule that affects its absorption maximum while inducing essentially no dipole moment. The spectral shift for this pool is due to the induction of higher multipoles. These also account for discrepancies that arise when one attempts to account quantitatively for available experimental results on carotenoid band shifts in terms of classical electrochromic theory.

INTRODUCTION

Carotenoid molecules embedded in the membrane of chloroplasts and photosynthetic bacteria have been used extensively as probes of membrane potential (for recent reviews see references 1 and 2). Their absorption spectrum is shifted instantaneously in response to an electric field, and this has made it possible to monitor the kinetics of light-induced potential changes in vivo. Because of the important applications of the carotenoid band shift, considerable effort has been invested in understanding the mechanism of its generation. As will be discussed below, a plausible interpretation now exists in terms of classical electrochromic theory. In this paper we provide a molecular description of the carotenoid band shift by carrying out quantum-mechanical calculations of carotenoid spectra in simulated protein environments. The major advantage of this approach over the classical one is that it leads to an interpretation of carotenoid band shifts in terms of specific molecular interactions. This in turn can both extend the ultimate utility of the method and, as we will show, permits an explanation of a number of phenomena not easily understood in terms of the classical theory.

Electrochromism, as defined by Liptay (3), involves changes in optical absorption induced by an external electric field. However, these changes may be due to a number of factors for molecules in a fixed orientation. The primary factor is the differential interaction of the ground and excited state with the external field. The frequency shift (in cm^{-1}), $\Delta\nu$, associated with the electronic transition is then given by

$$\Delta\nu = \frac{1}{hc}\left(-\Delta\mu \cdot \vec{F} - \frac{1}{2}\Delta\alpha F^2\right). \quad (1)$$

$\Delta\nu$ and $\Delta\alpha$ denote, respectively, the difference between ground- and excited-state dipole moments and polarizabilities ($\Delta\mu = \mu_e - \mu g$; $\Delta\alpha = \alpha_e - \alpha_g$) and $\vec{F}$ is the electric field.

It is evident that molecules such as carotenes, which have no dipole moment, should exhibit a quadratic response of ν to the field. Molecules with permanent dipoles will of course exhibit a linear response. As expected, in vitro experiments on carotenoids do produce a quadratic response (4, 5). Surprisingly, however, the electrochromic response of carotenoids in vivo has been found to be linear in all cases that have been studied. This result has been explained by assuming that carotenoids in vivo are exposed to a local electric field that is much stronger than the light-induced field (6). The effect of the local field is to induce dipoles in the carotenoid that then give rise to a linear shift in the presence of a weak external field (4, 5).

The source of the permanent local field is unclear. Reich and co-workers (7) have suggested that it originates in the Mg^{+2} ion of chlorophyll, which is complexed to a hydroxyl group of the carotenoid lutein. However, in photosynthetic bacteria where the nonpolar neurosporene is the primary carotenoid, such specific complexation is unlikely. Of

Dr. Kakitani's and Dr. Honig's present address is Department of Biochemistry, Columbia University, New York 10032

course, the chlorophyll could still be a source of the polarizing permanent field, but the many charged and polar amino-acid residues in the carotenoid binding proteins could serve equally well. Indeed there is considerable precedent for invoking the presence of charged amino acids to account for unusual spectral phenomena in carotenoid molecules. Recently it has been shown that a negatively charged residue in the vicinity of the 11-*cis* retinal chromophore of bovine rhodopsin is responsible for the 2,500 cm^{-1} (~50 nm) wavelength shifts that produce its in vivo absorption maximum (8). Similarly, a negatively charged residue located in a different position with respect to the all-*trans* chromophore of bacteriorhodopsin induces the even larger spectral shifts in bacteriorhodopsin (9). Charged amino acids have also been implicated in the energy storage mechanism of both pigments (10).

To quantitatively account for the spectral shifts in visible pigments and bacteriorhodopsin it was necessary to place the charged residues in close contact with the retinal chromophore. Because similar positioning of the external charge must be made for carotenoids, as we will show, the assumption of a constant local field is not valid and, as a result, simple electrochromic theory breaks down. This leads to a number of deficiencies in the interpretation of experimental results. It is not, for example, possible to attribute the source of the field to geometrically defined molecular interactions. This facility would be of considerable interest in cases where carotenoid shifts may be used to detect charge transfer events among reaction center components (see, e.g., reference 11).

Another difficulty with simple electrochromic theory is its inability to account for apparent inconsistencies between the magnitude of the spectral shift induced by the postulated local field and that produced by the transmembrane potential. According to classical theory, if a nonpolar molecule is exposed to a local field, a dipole moment will be induced in the ground and excited states. There should also be a concomitant change in transition energy that is quadratic with the magnitude of the field. The ~20-nm shift in absorption maximum of carotenoids in vivo relative to, say, their absorption in different solvents, has been attributed to the effects of the strong local field. However, in many systems there appear to be two pools of carotenoids, one that responds linearly to the external field and one that does not (12–18). One would expect then that the former pool would be red shifted with respect to the solution spectrum and the second pool would exhibit absorption spectra nearly identical to those observed in vitro.

This expectation is realized in some, but not all cases. In spinach chloroplasts De Grooth et al. (19) have shown that the pool of carotenoids that responds to the field is red shifted by ~20 nm relative to their in vitro spectrum while the pool that does not respond shows no wavelength shifts due to a permanent in vivo field. In contrast, the nonresponding pool is shifted by ~20 nm in G1C mutants of *Rhodopseudomonas sphaeroides* (12–15). As expected, the responding pool is further redshifted by 5–7 nm. However, the large red shift of the nonresponding pool appears inconsistent with classical theory. Conceivably, the nonresponding pool could be in a completely parallel orientation with respect to the plane of the membrane, thus accounting for the absence of an electrochromic response. However, this seems not to be the case in *Rps. sphaeroides* (20, 21), so other explanations must be sought.

To address these questions we report here semiempirical quantum-mechanical calculations of carotenoid absorption spectra in the field of local external charges. Our methodology, similar to that used in previous studies of visual pigments (8, 9), is summarized in the next section. We then attempt to account for the in vivo spectral shifts in terms of the specific positioning of an external charge with respect to the carotenoid. Having determined appropriate locations for the strong permanent field arising from this charge, we then consider the magnitude of the electrochromic response under different conditions.

We should point out here that the shortcomings in simple electrochromic theory arise when it is assumed that a constant electric field can be used to represent the interaction of a molecule with a strong local field. As we will show, the local field is highly variable over the dimensions of the molecule and in addition is strong enough to require that it be included explicitly in a quantum-mechanical treatment of molecular properties. However, the major deficiency of simple electrochromic theory is not the absence of quantum mechanics but rather the use of a constant field. When we use the term "classical electrochromic theory" in this work we will be referring to both assumptions. Our own treatment is, then, a quantum-mechanical description of "electrochromism" resulting from a strong variable electric field. We will use $\Delta\nu^1$ to denote the frequency shift resulting from a constant field due to the trans-membrane potential while $\Delta\nu$ will denote the shift due to a strong, local, permanent field.

METHODS

The visible and near-UV absorption spectra of linear polyenes such as carotenoids arise from transitions of electrons from occupied to unoccupied π orbitals of the molecule. π electron theory that treats the π orbitals explicitly involves a series of simplifying assumptions that make it possible to carry out quantum-mechanical calculations on large molecules containing π electron systems. Basically, one assumes a molecular geometry and then obtains wave functions, energy levels, etc. in terms of a set of integrals that are treated as parameters to be fit from experimental data (see, e.g., reference 22). The Pariser-Parr-Pople (PPP) method that is widely used can, with appropriate parameters, yield remarkably accurate results for a large set of molecular properties. In particular, spectroscopic transitions energy can often be obtained with close to experimental accuracy (see reference 23). The only significant modification we have made to PPP theory in this work involves the specific inclusion of a point charge and a constant electric field in our quantum-mechanical treatment (8, 23).

The visible absorption spectra of carotenoids are dominated by a single strong electronic transition. The three or more bands usually observed

involve transitions to different vibrational levels of the same electronic state. Our calculations produce only a single transition energy corresponding to the ground-excited state electronic energy difference at the ground-state geometry. For a broad structureless band, this should correspond to the absorption maximum (λ_{max}). When vibrational structure is evident, as is the case for many carotenoids, the calculated transition energy corresponds to the most intense vibrational component; when two lines are of equal intensity comparisons should be made to their mean.

Calculations were carried out on the π electron systems of the carotenoids shown in Fig. 1. The calculations differ from those published previously (23) only in our present use of a linear dependence of the resonance integral β on the bond length R. The relationship given by Roos and Sckancke (24) $\beta_{c-c} = -2.43 + 3.21\ (R_{c-c} - 2.40)$ was used for carbon—carbon bonds and $\beta_{c-o} = -2.74 + 4.21\ (R_{c-o} - 1.20)$ was used for carbon—oxygen bonds. Setting these parameters yields improved agreement with experiment for carotenoid absorption maxima. A distance-dependent dielectric constant given by $\epsilon = r - 2$ was used in the calculation of the coulombic interaction of atomic centers in the molecule with the external charge (8). The electric field was introduced into the Hamiltonian through the term $\Sigma_i e_i \vec{r}_i \cdot \vec{F}$, where e_i and $\vec{r}_i$ are, respectively, the charge and position vector of the atomic center i.

For calculations on neurosporene (Fig. 1) it was assumed that the π system is planar. Bond lengths were varied as described previously and bond angles were fixed at 120°. For lutein (Fig. 1), which has a conjugated double bond in one of its rings, a torsional ring—chain angle of 40° was assumed. This is a representative value taken from closely related molecules where steric hindrance distorts the π system (25). As mentioned above, the semiempirical parameters used in these calculations were chosen so as to produce reasonable absorption maxima for carotenoids. The calculated λ_{max} for lutein is 465 nm, which compares well with the experimental value in benzene of 460 nm (5). For neurosporene, comparison is more difficult because the first two vibrational lines are of approximately equal intensity (13). Thus, the calculated absorption maximum of 456 nm should, most appropriately, be compared to the mean of the two lines, which is 452 nm.

A more sensitive test of the theory is its ability to reproduce experimentally determined polarizabilities and dipole moments. Reich and Sewe (4) have determined for all-*trans*-β-*apo*-8'-carotenoic acid that the dipole moment difference between the ground and excited state is 10.7 Debye (D). Our calculated value of 9.9 D is thus in excellent agreement with experiment.

To obtain a value for ground-excited-state polarizability difference, $\Delta\alpha$, we calculated transition energies of various carotenoids in the presence of a constant electric field. Classical theory predicts a straight-line relationship between $\Delta\nu$ and $F^{2NE}{}_{11}$ ($h\nu = h\nu_0 - \frac{1}{2}\Delta\alpha_{11}F_{11}^2$) where $\Delta\alpha_{11}$ and F_{11} are components of the polarizability difference and electric field, respectively, along the long axis of the molecule. The expected straight-line relationship (see, e.g., Fig. 2 for neurosporene) was obtained for all molecules up to high values of the field, thus showing that our quantum-mechanical calculations reduce to simple electrochromic theory for small constant fields. The slope of the plot of ν vs. F_{11}^2 yields the theoretical value for $\Delta\alpha_{11}$. For lutein (Fig. 1*a*), bixindimethylester (Fig. 1*c*) and crocetindemethylester (Fig. 1*d*) we find values of 653 Å^3, 891 Å^2 and 634 Å^3, which should be compared with the experimental values of 1,070; 1,550; and 780 Å^3 (26), respectively. Clearly the quantitative agreement is not good, with the calculated values ranging from 60–80% of the experimental ones. However, qualitatively, we obtain values in the proper range, and this is all that is required for the part of our analysis that makes use of calculated polarizabilities. The calculated value of $\Delta\alpha_{11}$ for neurosporene, which can be obtained directly from Fig. 2, is 585 Å^3. This is reasonably close to the experimental value for crocetindimethylester (780 Å^3), which has been used previously as an estimate for neurosporene (15). The calculations summarized in Fig. 2 differ from those used in the rest of this work in that no external charges were included and as a result, the molecule had no permanent dipole moment. All the calculations reported below included an external charge which induced a permanent dipole in neurosporene.

RESULTS

Our first goal was to determine the positions of the external charges that reproduce the ~20-nm shifts in absorption due to the permanent local fields. A point charge was placed in different positions around the π system of neurosporene, as shown in Fig. 3, and absorption maxima

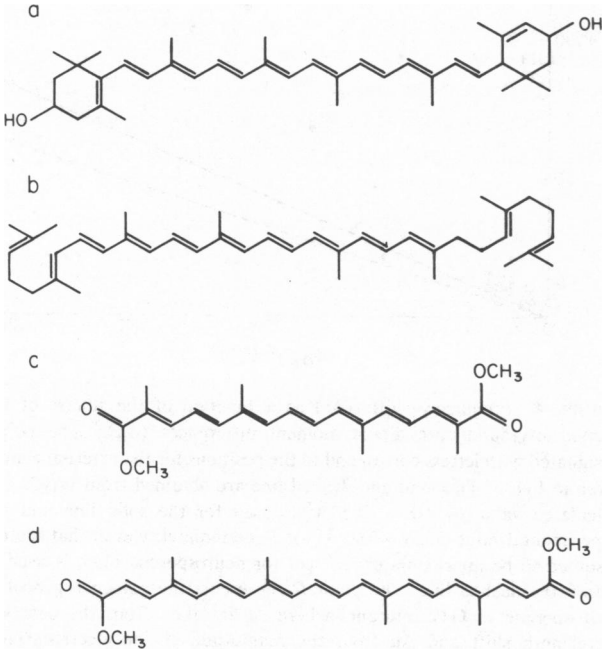

FIGURE 1 Structural formulas. (*a*) Lutein, (*b*) neurosporene, (*c*) bixindimethylester, (*d*) crocetindimethylester.

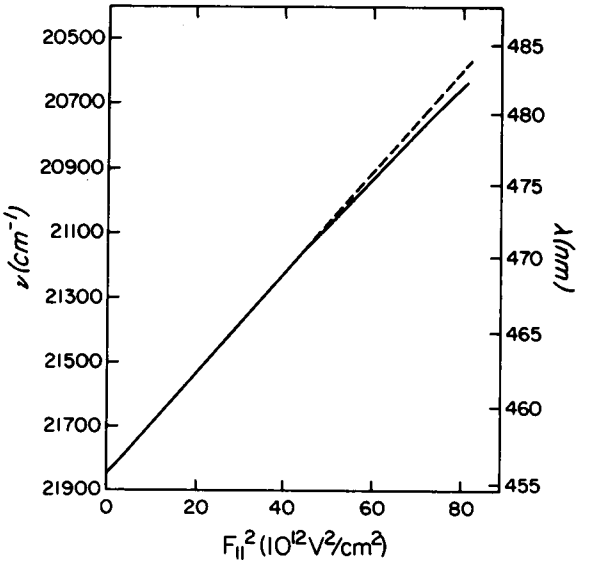

FIGURE 2 Plot of the calculated frequency shift ($\Delta\nu$) in neurosporene vs. F_{11}^2. No external charge is included in these calculations. The dashed line is an extension of the line defined by the initial slope.

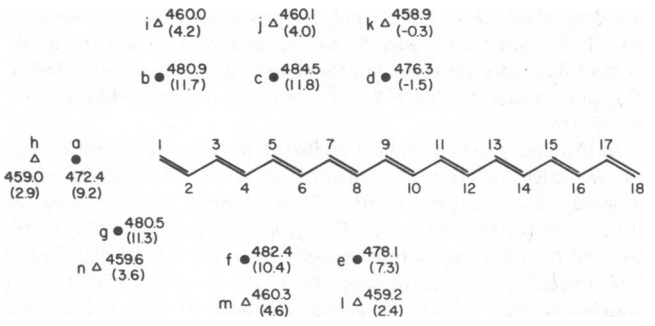

FIGURE 3 π electron system of neurosporene with various positions for an external negative charge denoted by lower case letters. Numbers besides each letter correspond to calculated adsorption maxima. Numbers in parenthesis are the difference in dipole moment between the ground and excited state ($\Delta\mu$) induced by the external change. ●, positions for the charge 3.7 Å from the nearest atom. Δ, positions 6 Å from the nearest atom.

were calculated. As is evident from the figure, there are many locations for the charge that reproduce the correct shift. However, in all of these the external charge must be placed in van der Waals contact with the chromophore. The local field so induced is extremely large (assuming a dielectric constant of 2 it is $\sim 5 \times 10^7$ V/cm for an atom in contact with the charge, and is reduced to $\sim 5 \times 10^6$ V/cm for an atom 10 Å further down the chain). Our results suggest, then, that carotenoids that exhibit large in vivo wavelength shifts must be quite close to one or more charged amino acids or polar groups. Because charged amino acids are likely to be paired with another charge of opposite sign, it is necessary to assume that the resulting dipole is oriented so that one end is much closer to the chromophore than the other. Otherwise, the effects of the two charges would cancel. Fig. 3 implicitly assumes that the dominant interaction is with the proximal member of the charge pair.

We wish to emphasize here that our calculations in no way imply that a single charge or dipole is responsible for the wavelength shift and linear electrochromic response of carotenoids in vivo. Rather, we are assuming the simplest possible model in order to investigate the possible insights arising from a quantum mechanical description of the electrochromic response. One question of interest concerns the extent to which a strong constant electric field can be used to represent the effects of a point charge, that is, what errors are introduced by assuming that classical electrochromic theory can be used to represent the permanent electric field. We first test the self-consistency of this assumption by calculating the induced dipole moment difference along the molecular axis, $\Delta\mu_{11}$, using two different methods.

According to classical theory, $hc\Delta\nu = -\frac{1}{2}\Delta\alpha_{11} F_{11}^2$ and $\Delta\mu_{11} = \Delta\alpha_{11} F_{11}$. Combining the two equations we have

$$\Delta\nu = -\frac{\Delta\mu_{11}^2}{2hc\Delta\alpha_{11}}. \qquad (2)$$

This equation states that the frequency shift induced by an electric field is proportional to the square of the induced dipole moment. The two straight lines in Fig. 4 are plots of Eq. 2 using our calculated value for neurosporene of $\Delta\alpha$ = 585 Å^3 (see Fig. 2) and the value of 780 Å^3 used by Symons et al. (15) in their analysis.

We now show that the experimental results for the *Rps. sphaeroides* G1C mutant lead to a contradiction if the straight-line relationship indicated by classical electrochromic theory is used to estimate $\Delta\mu$. The frequency shift ($\Delta\nu$) of the carotenoid pool that responds to the permanent field in G1C is ~1,100 cm^{-1} (25 nm). On the dashed line of Fig. 4 this corresponds to a value of $\Delta\mu$ of ~18.5 D. We can obtain an independent estimate for $\Delta\mu$ from the magnitude of the electrochromic response to the trans-membrane potential that was found by Symons et al. (15), to be 137 mV/nm shift. (Others [8–10] have found smaller shifts, the use of which would, as will become evident, only strengthen the argument.) Using an angle of 45° for the orientation of the carotenoid molecules with respect to the membrane (22) the relationship $hc\Delta\nu^1 = \Delta\mu_{11}F_{11}$ predicts a value of 12.2 D for $\Delta\mu_{11}$ (or smaller, if smaller values for the electrochromic response are used). Comparing this with the value of $\Delta\mu_{11}$ = 18.5 D required to account for the 25-nm shift due to the permanent field, it is apparent that the two numbers, both obtained from classical theory, are in poor agreement.

The value of 12.2 D is more reasonable as classical

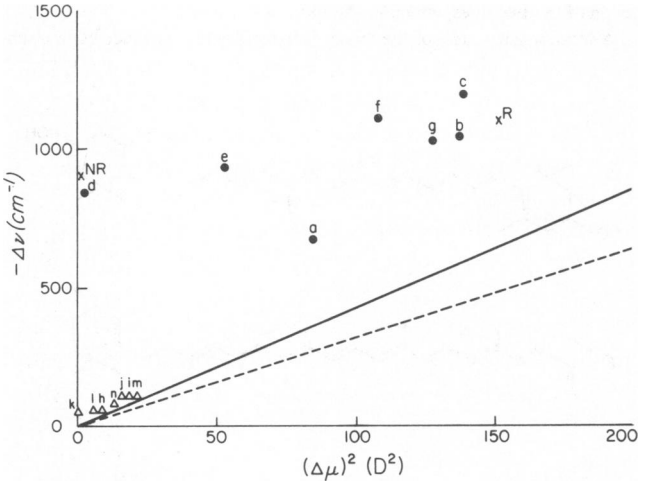

FIGURE 4 Frequency shifts ($\Delta\nu$) as a function of the square of the excited-to-ground-state dipole moment differences ($\Delta\mu$).^{2} The points designated with letters correspond to the positions for the external charge given in Fig. 3. The solid and dashed line are obtained from Eq. 2. The calculated value of $\Delta\alpha$ = 585 Å^3 is used for the solid line and the experimental value of $\Delta\alpha$ = 780 Å^3 for crocetindimethylester that is often assumed to be approximately correct for neurosporene (11), is used to obtain the dashed line. The point *R* represents the responding pool of neurosporene in G1C mutants where $\Delta\nu$ is taken from the observed wavelength shift and $\Delta\mu$ from the magnitude of the electrochromic response found by Symons et al. (11). The point NR corresponds to the nonresponding neurosporene pool where $\Delta\mu$ is assumed to be zero because no electrochromic response is observed.

theory is certainly valid for the transmembrane potential. The experimental point, indicated on Fig. 4 as *R*, uses this value for $\Delta\mu_{11}$ and the experimental value of 25 nm (~1,100 cm^{-1}) for $\Delta\nu$. Another way of describing the discrepancy discussed above is to note the deviation of the experimentally determined point (*R*) from the straight lines that are based on Eq. 2. Clearly Eq. 2 is not valid.

We can determine another experimental point from the nonresponding pool of carotenoids. These show no electrochromic response, so $\Delta\mu = 0$. On the other hand they are shifted by ~900 cm^{-1} relative to their solution absorption spectra. The point corresponding to these values of $\Delta\mu$ and $\Delta\nu$ is designated *NR* on Fig. 4. The deviation from Eq. 2 is obviously quite dramatic.

Clearly, for both the responding (*R*) and nonresponding (*NR*) pool of carotenoids, the observed frequency shift due to the permanent field is much larger than expected from Eq. 2, which uses the magnitude of the electrochromic response to determine $\Delta\nu$. The discrepancy results from the breakdown of classical theory when large and variable fields are involved. This may also be seen clearly from the calculated points (*a–m*, see Fig. 3) given in Fig. 4 that show large deviations from the solid line, which is based on classical theory (though it uses our calculated $\Delta\alpha$). Notice that for weaker fields, corresponding to point charges relatively far away from the carotenoid, agreement with classical theory is excellent. It is also worth pointing out that the experimental point *R* corresponds quite well with a number of possible positions for the external charge. It appears that our simple model of a single charge which determines both $\Delta\nu$ and $\Delta\mu$ succeeds quite well in accounting for both the constant wavelength shift ($\Delta\nu$) and the electrochromic shift ($\Delta\nu^1$ of the carotenoid pool that responds to the transmembrane potential.

It is of interest to consider the source of the breakdown in classical theory. Fig. 4 shows that, in general, the calculated frequency shifts are larger than those expected from the $\vec{\mu} \cdot \vec{F}$ interaction Eq. 2. Our analysis indicates that the additional shift is due to quadrupoles and higher multiples induced by the external charge. As two examples, we consider charge distribution induced in neurosporene by a negative charge placed near the end and middle of the chromophore (point *b* and *d*, respectively). Qualitatively, one expects positive charge to be built up near the external negative charge, and negative charge to be distributed at other points along the chain. This should result in a primarily dipolar distribution for point *b* and a quadrupolar distribution for point *d*. This general pattern is evident from the calculated values of the excited-ground state charge densities given in Fig. 5. A net increment of positive charge is built up in the vicinity of the external negative charge although the actual charge distribution is fairly complex. In any case, it is clear that higher multipoles are induced; it is the interaction of these moments with the variable field that is responsible for deviations from the linear $\Delta\nu$ vs. $\Delta\mu^2$ relationship expected from classic theory.

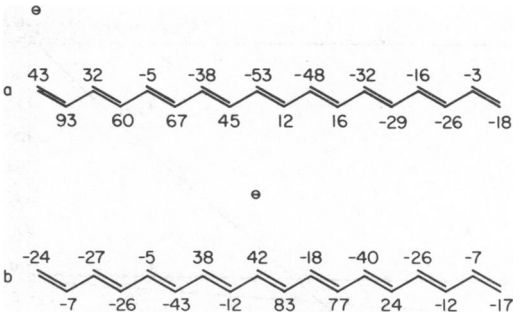

FIGURE 5 Calculated excited-ground charge distribution in neurosporene induced by an external charge.

Because these moments do not contribute to the electrochromic response ($\Delta\nu^1$ but do effect $\Delta\nu$), they are responsible for the breakdown of Eq. 2, which assumes that both $\Delta\nu$ and $\Delta\nu^1$ arise totally from $\Delta\mu$.

The existence of induced quadrupole moments provides an explanation for the absence of an electrochromic response in carotenoids that show relatively large (~20 nm) shifts due to the permanent field. Taking point *d* in Figs. 3 and 4 as an example, note that a ~20-nm shift is produced by a charge located near the middle of the carotenoid even though $\Delta\mu$ is only -1.3 Debye. Because the interaction with the transmembrane potential is only through the $\Delta\mu$ term the electrochromic response will be negligible. Thus we find that it is possible to obtain a large permanent wavelength shift due to an induced quadrupole while the electrochromic response is small. This can account for the large $\Delta\nu$ (~900 cm^{-1}) exhibited by the nonresponding carotenoid pool. The close proximity of position *d* in Fig. 4 to the experimental point for this pool (*NR* in Fig. 4), though undoubtedly fortuitous, is certainly suggestive.

Finally we consider the electrochromic response of neurosporene exposed to an external charge fixed at position *b*. As can be seen from Fig. 4, this position is one of a number of possibilities that succeeds in reproducing both the experimental $\Delta\nu$ as determined from the permanent absorption shift, and the experimental $\Delta\mu$ as determined from the electrochromic response to the transmembrane potential. We carried out calculations on neurosporene exposed to both a fixed charge at position *b*, and to a constant electric field. Fig. 6 describes how the various molecular properties are influenced by constant field due to the transmembrane potential. First, it is evident that the ground-excited state dipole moments are only marginally affected by the field, with $\Delta\mu$ varying by ~5% as F_{11} goes from 0 to 10^5 V/cm. The oscillator strength *f* decreases by an even smaller amount for the same variation in field strength and thus cannot be responsible for the observed electrochromism. The same conclusion has been reached previously (15). It is apparent then, in keeping with classical theory, that the overwhelming determinant of the electrochromic response is the linear variation of λ with

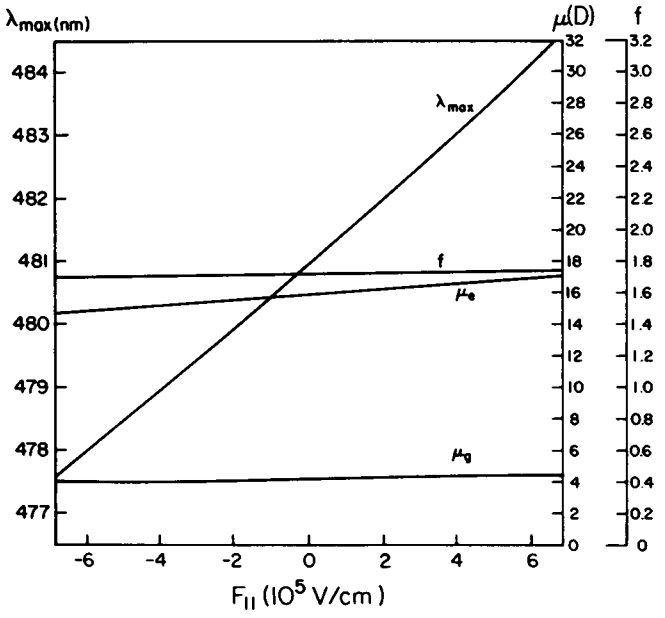

FIGURE 6 The calculated electrochromic response of neurosporene with an external charge at position *b* in Fig. 3. Absorption maxima (λ_{max}), ground- and excited-state dipole moments (μ_g and μ_e), and oscillator strengths (f) are plotted as a function of constant electric field. The point differs from Fig. 2 in that an external charge and an electric field are included in the quantum-mechanical calculations.

$F_{||}$. Moreover, using the tilt angle of neurosporene with respect to the plane of the membrane of 45° (21) and assuming a membrane thickness of 50 Å, we calculated an electrochromic response of 133 mV/nm which is in close agreement with the 137 mV/nm result of Symons et al. (15).

DISCUSSION

We have seen that classical theory provides an excellent description of the electrochromic response of carotenoids, if it is assumed, as originally suggested by Reich and co-workers (4–6), that a dipole is induced in the responding carotenoids by a strong local electric field. In this paper we have shown that a quantum-mechanical treatment is required for a quantitative description of the strong permanent field. The model we have used assumes that this field results from a single point charge in the vicinity of the carotenoid. Before commenting on the implications of the model it is worth discussing the reliability of the calculations upon which it is based.

Our work on model compounds summarized in the Methods section indicates that the molecular orbital approach we used accounts quite well for the relevant properties ($\Delta\alpha$, $\Delta\nu$, λ) of the isolated molecules. The quantitative reliability of our treatment of the external point charge is less certain, and as a result the dependence of $\Delta\nu$ and $\Delta\mu$ on the position of the charge, as given in Fig. 4, is likely to be only qualitatively correct. Nevertheless, internally consistent and physically reasonable features of our calculations lend them an enhanced credibility. First, it is reassuring that positions for the charge that produce 25-nm red shifts also produce values of $\Delta\mu$ (~12 D) that account for the magnitude of the electrochromic response. Second, the possible locations we find for the charges place them in van der Waals contact with the carotenoid. Smaller distances would of course be physically unacceptable while charges much further away would be unlikely to be effective.

Previous work on visual pigments (8) and bacteriorhodopsin (9) identified charged groups that are also in van der Waals contact with a chromophore responsible for the color of these pigments. The much larger wavelength shifts in these retinal-containing pigments, relative to the carotenoids, are due to their cationic character (23). It is perhaps somewhat disconcerting to postulate a charged species in close contact with the hydrophobic chain of the carotenoid in the low dielectric medium of the membrane. However, there is strong evidence for the existence of related interactions in the retinal containing pigments that are also embedded in membranes. Apparently, charges or charge pairs can be stabilized by hydrogen bonding to other polar groups on the protein that provide a form of internal solvation.

It is important to emphasize that our calculations are based on a purely phenomenological model that represents the strong local field postulated by Reich et al. (4) in the form of a single point charge. It is possible that a number of polar but neutral functional groups appropriately positioned and oriented could provide a field of equivalent strength. Nevertheless, a single point charge in van der Waals contact with a molecule generates an enormous local field and thus places limits on acceptable molecular models for the interaction. It would be extremely interesting in this regard to calculate the magnitude of the wavelength shifts induced by the interactions of carotenoids with chlorophylls. In one case, the specific complexation of lutein with chlorophyls *a* and *b*, experimental data are available (5). No direct interactions between chlorophylls and nonpolar carotenoids are found in vitro; however, this by no means precludes significant effects in vivo. On the other hand, there is no a priori reason to suspect that the local electric field is due to chlorophylls; charged amino acids could be at least as effective.

A quantitative treatment of the electrostatic interaction of charged species with carotenoids allows one, in principle, to extract useful geometric information from induced wavelength shifts. For example, Cogdell et al. (11) have found that *Rps. sphaeroides* reaction centers can induce both red and blue shifts in bound carotenoid, the direction of the response depending on the distribution of charge among the components of the reaction center. Given the orientation of the carotenoid with respect to the reaction center components, careful analysis of spectral shifts might allow one to determine the distances between the various molecules.

Finally, we summarize the major conclusions of this work:

(*a*) A quantum-mechanical treatment is required for a quantitative description of the spectroscopic effects of a strong local field on carotenoid molecules.

(*b*) Depending on the placement of the field, dipoles, quadrupoles, or higher multipoles may be induced in the carotenoid.

(*c*) The magnitude of the electrochromic response to the transmembrane potential depends only on the interaction of the dipolar term with the field. Thus, the correlation of $\Delta\mu$ with $\Delta\nu$ expected on the basis of classical electrochromic theory is not experimentally or theoretically observed.

(*d*) Placement of an external charge near the center of the carotenoid induces almost no dipole, but a large quadrupole moment is induced. It is possible in this case to see a large spectral red shift but no electrochromic response. This provides a possible explanation of the non-responding pool of red-shifted carotenoids in *Rps. sphaeroides*.

(*e*) A charge located near the end of neurosporene can quantitatively account for the observed (in vitro → in vivo) 25-nm wavelength shift and for the magnitude of the electrochromic response to the transmembrane potential.

(*f*) Once an induced $\Delta\mu$ is assumed, the assumptions of classical electrochromic theory are valid.

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REFERENCES

1. Reich, R. 1979. Intrinsic probes of charge separation. *In* Light-induced Charge Separation in Biology and Chemistry. H. Gerischer and J. J. Katz, editors. Berlin Dahlem Konferenzen. 361–387.
2. Cramer, W., and A. R. Crofts. 1982. Photosynthesis: Energy Conversion by Plants and Bacteria. Govindjee, editor. Academic Press, Inc., N.Y. In press.
3. Liptay, W. 1969. Elektrochromie-solvatochromie. *Angew. Chem.* 81:195–232 (*Int. Ed. Engl.* 8:177–188).
4. Reich, R., and K.-U. Sewe. 1977. The effect of molecular polarization on the electrochromism of carotenoids. I. The influence of a carboxylic group. *Photochem. Photobiol.* 26:11–17.
5. Sewe, K.-U., and R. Reich. 1977. The effect of molecular polarization on the electrochromism of carotenoids. II. Lutein-chlorophyll complexes: the origin of the field-indicating absorption change at 520 nm in the membranes of photosynthesis. *Z. Naturforsch. Sect. C. Biosci.* 32:161–177.
6. Reich, R., and S. Schmidt. 1972. Uber den Einbluβ elektrischer Felder auf das Absorptionsspektrum von Farbstoffmolekülen in Lipidschichten. I. Theorie. *Ber. Bunsen. Ges. Phys. Chem.* 76:589–598.
7. Sewe, K.-U., and R. Reich. 1977. Influence of chlorophylls on the electrochromism of carotenoids in the membranes of photosynthesis. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 80:30–34.
8. Honig, B., U. Dinur, K. Nakanishi, V. Balogh-Nair, M. A. Gawinowicz, M. Arnaboldi, and M. G. Motto. 1979. An external point charge model for wavelength regulation in visual pigments. *J. Am. Chem. Soc.* 101:7084–7086.
9. Nakanishi, K., V. Balogh-Nair, M. Arnaboldi, K. Tsujimoto, and B. Honig. 1980. *J. Am. Chem. Soc.* 102:7945–7957.
10. Honig, B., T. Ebrey, R. H. Callender, U. Dinur, and M. Ottolenghi. 1979. Photoisomerization, energy storage and charge separation: a model for light energy transduction in visual pigments and bacteriorhodopsin. *Proc. Nat. Acad. Sci. U.S.A.* 76:2503–2507.
11. Cogdell, R. J., S. Celis, H. Celis, and A. R. Crofts. 1977. Reaction center carotenoid band shifts. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 80:190–193.
12. De Grooth, B. G., and J. Amesz. 1977. Electrochromic absorbance changes of photosynthetic pigments in *Rhodopseudomonas sphaeroides*. II. Analysis of the band shifts of carotenoid and bacteriochlorophyll. *Biochim. Biophys. Acta.* 462:247–258.
13. Holmes, N. G., and A. R. Crofts. 1977. The carotenoid shift in *Rhodopsuedomonas sphaeroides*. The flash-induced change. *Biochim. Biophys. Acta.* 459:492–505.
14. Holmes, N. G., and A. R. Crofts. 1977. the carotenoid shift in *Rhodopseudomonas sphaeroides*. Change induced under continuous illumination. *Biochim. Biophys. Acta.* 461:141–150.
15. Symons, M., C. Swysen, and C. Sybesma. 1977. The light-induced carotenoid absorbance changes in *Rhodopseudomonas sphaeroides*. An analysis and interpretation of the band shifts. *Biochim. Biophys. Acta.* 462:706–717.
16. Holmes, N. G., C. N. Hunter, R. A. Niederman and A. R. Crofts. 1980. Identification of the pigment pool responsible for the flash-induced carotenoid band shift in *Rhodopseudomonas sphaeroides chromatophores. FEBS (Fed. Eur. Biochem. Soc.) Lett.* 115:43–48.
17. Matsuura, K., T. Ishikawa, and M. Nishimura. 1980. Correlation of Membrane-potential-sensing carotenoid to pigment-protein complex II in *Rhodopseudomonas sphaeroides. Biochim. Biophys. Acta.* 590:339–344.
18. Webster, G. D., R. J. Cogdell, and J. G. Lindsay. 1980. Identification of the carotenoid present in the B-800-850 antenna complex from *Rhodopseudomonas capsula* as that which responds electrochromically to transmembrane electric fields. *Biochim. Biophys. Acta.* 591:321–330.
19. De Grooth, B. G., H. J. Van Gorkom, and R. C. Meiburg. 1980. Electrochromic absorbance changes in spinach chloroplasts induced by an external electric field. *Biochim. Biophys. Acta.* 389:299–314.
20. De Grooth, B. G. 1980. Ph.D. Dissertation. University of Leiden. 73.
21. Breton, J. 1974. A linear dichroism study of pigment orientation in photosynthetic bacteria. *Biochem. Biophys. Res. Commun.* 59:1011–1017.
22. Salem, L. 1960. Molecular Orbital Theory of Conjugated Systems. The Benjamin Co., Inc., New York.
23. Honig, B., A. Greenberg, U. Dinur, and T. G. Ebrey. 1976. Visual pigment spectra: implications of the protonotation of the retinal Schiff base. *Biochemistry.* 15:4593–4599.
24. Roos, B., and P. Sckancke. 1967. Semiempirical parameters in π-electron systems. *Acta. Chem. Scand.* 21:233–242.
25. Honig, B. 1978. Light energy transduction in visual pigments and bacteriorhodopsin. *Ann. Rev. Phys. Chem.* 29:31–57.
26. Labhart, H. 1961. Beeinflassung der Light absorption Organischen farbstaff durch äussere Elektrische Felder. *Helv. Chim. Acta.* 56:457–460.