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Visual Chromophore Electronic Structure*

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Abstract. The implications that recent work on the electronic structure of polyenes have for the low lying electronic states of retinal are reviewed and a very simple model for the diffuseness of the absorption spectra of visual chromophores is presented.

Key words: Retinal – Polyenes – Electronic structure.

In order to understand the process of vision on a microscopic scale, it is necessary to determine the nature of the interactions between opsin and retinal both before and after the absorption of a photon. This description in turn rests on a detailed description of the electronic states of the visual pigments and their behavior under perturbation. We have completed some spectroscopic studies which bear on this problem and would like to bring three points to the attention of this conference. The first is to remind workers in this area that the best evidence that we have to date says that the lowest energy strong absorption by retinal at 380 nm (or its Schiff base) derives from a transition to the *second* excited singlet state, not the first. Of course, this has important implications for the photochemistry of visual systems. Secondly, we would like to summarize the recent information on the electronic structure of retinal that we have developed from low resolution spectroscopy (linewidths greater than 200 cm^{-1}) including conclusions that can be drawn regarding the reason for the lack of vibrational structure, the assignments of the higher energy absorptions and the ground and excited state dipole moments. Finally, we would like to draw attention to the possibility of obtaining high resolution (linewidths less than 10 cm^{-1}) spectra directly for retinal and the visual pigments and report on our recent progress in this area. Due to the limitation of space in this short note, only the current picture of polyene electronic structure and our model for the observed broadening in retinyl species are included in the published note.

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1. Polyene Electronic Structure

Here it is reasonable to focus attention on the manifold of singlet states: retinyl Schiff bases and unsubstituted polyenes intersystem cross to the triplet manifold only very inefficiently [1]. There is now direct high resolution spectroscopic data in the literature which confirms that the lowest energy excited singlet state for diphenyloctatetraene [2, 3], 1,10-dimethylundecapentaene [4], and 1,12-dimethyltridecahexaene [5] is, to first order, forbidden in absorption from the ground state. The strong visible absorption band that is observed for these substances derives from a transition to the second excited singlet state. The indirect argument for the presence of a forbidden singlet state just lower in energy than the state responsible for the strong visible absorption for all polyenes has been given in detail elsewhere [3, 6]. We briefly reproduce the essential features here.

The pre-1972 picture of polyene electronic structure is summarized in the left hand side of Figure 1. As for the particle in a box, the inversion symmetry of the energy ordered one-electron orbitals calculated for the linear polyenes by a variety of techniques (Huckel Theory, ppp-Theory etc.) alternate between even and odd. Since these systems contain an even number of electrons, the ground state configuration is necessarily even and the lowest, energy 1-electron excitation is necessarily odd with respect to inversion. Thus in this picture the transition to the lowest energy excited singlet state is expected to be strongly allowed. For strongly allowed transitions we can with reasonable confidence predict the fluorescence lifetime from the integrated absorption intensity [7]. Moreover, if the strong absorption and the fluorescent emission arise from the same excited electronic state, we expect there to be reasonable overlap between the absorption and emission spectra and should observe similar solvent shifts for both the absorption and emission.

In fact, the observed absorption and emission spectra of almost all polyenes including retinal are significantly at variance with these expectations (Fig. 2).

The strong absorption and the emission spectra of polyenes do not overlap. This is especially striking in those cases where there is resolved vibrational structure, for example in Figure 2 we note that there is a gap of 2000 cm^{-1} between the lowest

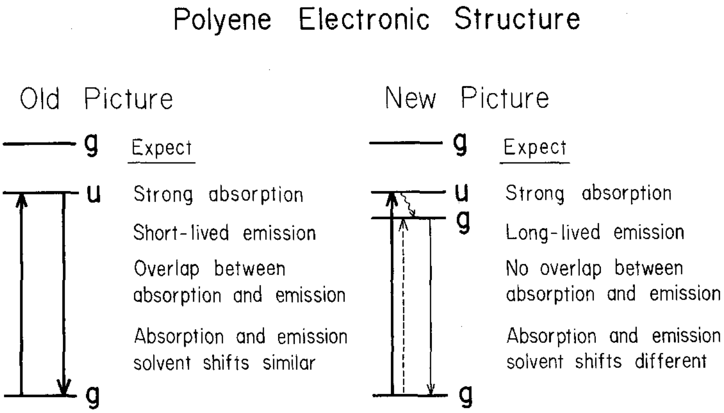


Fig. 1. Energy level diagrams for polyenes before and after 1972

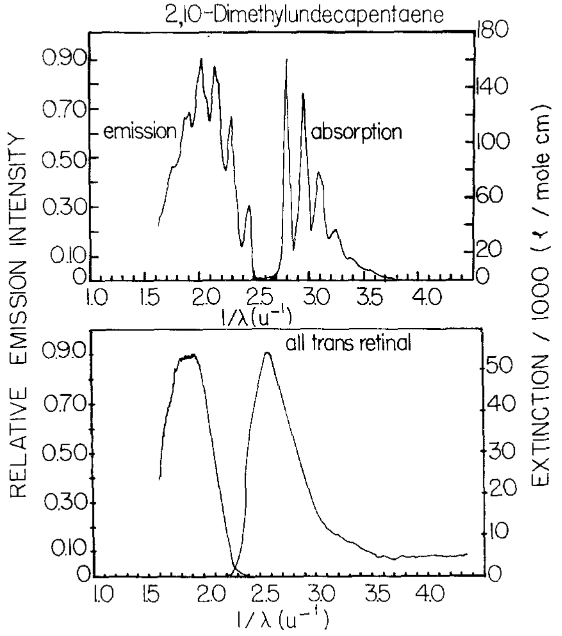


Fig. 2. Absorption and emission spectra for 2,10-dimethylundecapentaene and retinal in EPA at 77 K

energy peak in the absorption spectrum and the highest energy emission for undecapentaene. Attempts to explain this lack of overlap as the consequence of a large change in molecular geometry upon excitation using the Franck-Condon principal fails in the case of the resolved spectra. Franck-Condon forbidden electronic transitions normally exhibit long progressions in one or more vibrations with a smoothly varying intensity as expected on the basis of *any* model involving displaced smooth potential surfaces. As is seen in the pentaene spectrum in Figure 2, both absorption and emission show and abrupt onset followed by a smooth Franck-Condon envelope involving at most 3 or 4 vibrational quanta.

Integration of the strong absorption band and application of the lifetime-intensity relation of Einstein as modified for polyatomic species by Strickler and Berg [7] leads to the prediction of a very short fluorescence lifetime. In fact, the observed lifetime for a variety of polyenes is dramatically too long (Table 1).

Finally, when the local polarizability for a polyene is changed by changing the solvent environment, the absorption spectrum shifts by approximately 10^4 cm^{-1} per unit change in $\frac{n^2-1}{n^2+2}$ for all polyenes so far studied including retinal. The emission spectrum shifts by at most a few hundred cm^{-1} per unit polarizability change. These relations between absorption and emission were very puzzeling before 1972 when it was directly shown for a diphenylpolyene that the electronic structure was not as shown in the left hand side of Figure 1 but rather corresponded to the right hand side [2, 3]. The presence of a forbidden singlet state at lower energy than that responsible to the strong absorption completely rationalized the observed absorption and emission behavior of polyenes. The lack of overlap just reflected the fact that the emis-

Table 1. Representative polyene fluorescence data

Molecule[a]	Solvent[b]	Q	τ^{c} (ns)	τ_0^{d} (ns)	T_0^{e} (ns)	τ_0/T_0
Diphenyloctatetraene	Benzene	0.15	7.4	49	2.3	21[f]
	Cyclohexane	0.09	6.2	69	4.1	17[g]
Retinylacetate	MCH/IH	0.02	6.0	300	2.8	107[h]
Retinol	MCH/IH	0.02	4.7	235	2.7	87[i]
9-cis retinol	MCH/IH	0.007	6.6	940	3.4	276[h]
13-cis retinol	MCH/IH	0.01	6.0	600	3.4	176[h]

[a] All-trans unless noted

[b] MCH: methylcyclohexane; IH: isohexane

[c] The measured lifetime

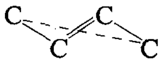
[d] $\tau_0 = \tau/Q$

[e] Calculated from the integrated absorption coefficient

[f] Birks, J. B., Dyson, D. J.: Proc. Roy. Soc. A **275**, 135 (1963)

[g] Berlman, I. B.: Handbook of fluorescence spectra of aromatic molecules, pp. 322, 323. New York: Academic Press 1971

[h] Dalle, J. P., Rosenberg, B.: Photochem. Photobiol. **12**, 151 (1970)

sion came from a different level than that responsible for the strong absorption, since the emitting state had a very small transition probability the long lifetime of the emission followed directly and, in fact, a quantitative correspondence between the emission lifetime and the gap between absorption and emission could be established [3]. The vanishing intensity of the absorption to this low lying level also quantitatively agreed with the very small solvent shifts observed for the fluorescence. A theoretical analysis of what modifications had to be made in the traditional picture of polyene electronic structure to obtain agreement with experiment was worked out by Schulten and Karplus [8]. The important conclusion from their work was that electron correlation was very important in these systems: consequently, molecular orbital treatment aimed at developing a single Slater-determinant description for the lowest energy excited state were doomed to failure. They also pointed out relatively simple descriptions derived from rather complete calculations for the lowest energy excited state in polyenes. If the energy ordered 1-electron π-orbitals for butadiene are labeled abcd, the lowest energy excited state may be described as roughly equal parts of the configuration $|a\bar{a}c\bar{c}|$ mixed with the superposition $|a\bar{a}b\bar{d}| + |ab\bar{b}\bar{c}|$. The simplest description comes in terms of valence bond theory where the lowest energy excited state is that with the connonical structure . This pronounced bond order reversal is a distinctive property of this symmetric excited state which obtains also for the longer polyenes [9].

Thus, although the direct evidence is as yet only in hand for model polyenes, the indirect evidence is quite conclusive – that retinal and its derivatives have a chemically quite distinct state just lower in energy than the state responsible for the strong u.v. absorption. In developing detailed microscopic pictures for the effects of perturbations on the absorption spectra and photochemistry of retinal, models that are incapable of correctly ordering the electronic states in simple polyenes should be used with great caution. The extent to which exciting new models may be developed is well testified to by the poster presentation of Klaus Schulten at this conference.

2. Spectral Diffuseness in Retinyl Compounds

Absorption and emission spectra for retinal, axerophtene, anhydrovitamina A and 2,10-dimethylundecapentaene in rigid glass matrixes have been obtained [10]. In this series of compounds the electronic structure of retinal is continuously simplified: the $n\pi^*$ states are removed in axerophtene, the ring-side chain angle is fixed in anhydrovitamin A and the β-ionylidene ring is completely removed in 2,10-dimethylundecapentaene. It was observed that retinal and axerophtene showed diffuse spectra both in absorption and emission while the spectra of anhydrovitamin A and 2,10-dimethylundecapentaene were structured. This was interpreted as indicating that the principal source of broadness in retinal's spectrum derived from a statistical distribution of conformers differing in the torsional angle between the β-ionylidene ring and the polyene side chain. While the ground state energy is but little affected by twisting about the 6—7 bond, the ^1B_u excited state energy changes a great deal. Hence, a wide range of transition energies are present in the experimental ensemble with the consequence being a complete loss of structure in the spectrum.

This model for the broadening can be quantified in a way that is both amusing and instructive. The best feature of this model is the fact that it nicely accounts for the fact that β-carotene, which has two β-ionylidene rings, has a structured absorption spectrum.

The key piece of information is shown in the left hand side of Figure 3 where the energy of the strongly allowed absorption is plotted as a function of the number of double bonds for a series of simple polyenes [11]. This curve is fit by the function $\bar{\nu} = (14500 + \frac{77400}{n})\ \text{cm}^{-1}$ where $\bar{\nu}$ is the excitation energy in wave numbers and n is

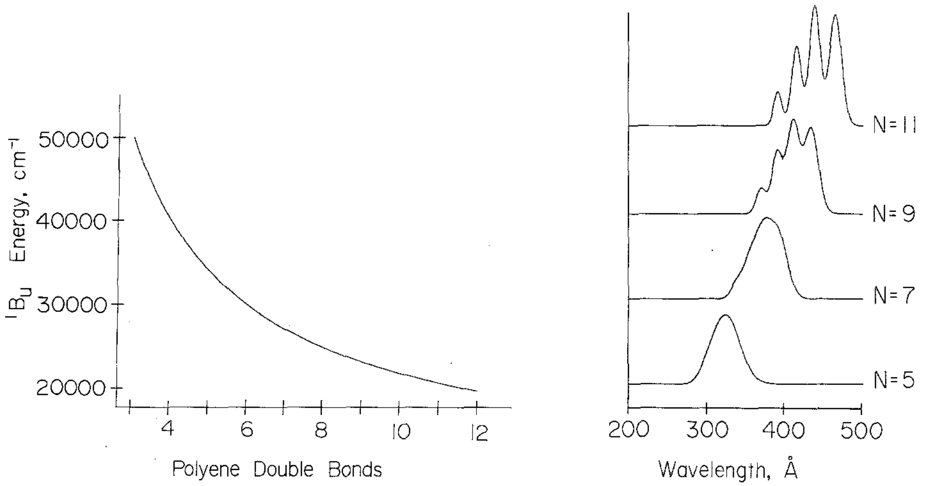


Fig. 3. Calculated dependence of transition energy (left) and bandwidth (right) for polyenes containing a β-ionylidene ring on the number of double bonds. The broadening is assumed to derive entirely from the distribution of ring-side chain angles which is taken to be the same for all molecules

the number of conjugated double bonds in the system. Twisting about the 6–7 bond in system containing a β-ionylidene ring may be associated with a decrease in n. Thus, the presence of a range of conformers gives a spread in excitation energies $\Delta\bar{\nu} = \frac{77400}{n^2}\Delta n\ \mathrm{cm}^{-1}$. We might guess that a distribution of conformers differing by the degree of twist about the 6–7 bond introduces a spread of roughly 1/2 in the number of double bonds. With this distribution in conformations, the excitation energy would be expected to exhibit a spread $\Delta\bar{\nu} = \frac{38700}{n^2}\ \mathrm{cm}^{-1}$. Simulated spectra generated by superposing Gaussians of this width with intensities 1.0, 1.1, 0.7, and 0.3 at $\bar{\nu}$, $\bar{\nu} + 1320$, $\bar{\nu} + 2640$, and $\bar{\nu} + 4120$, respectively are shown in the right hand side of Figure 3. $\bar{\nu}$ is the calculated ${}^1A_g \rightarrow {}^1B_u$ transition energy for a polyene containing a double bond and one β-ionylidene ring. The spectra built using this model are remarkably similar to those actually observed for caratenoids [12]. If this is taken to strongly suggest that the existence of a range of conformers differing in their ring-side chain torsional angle is in fact the source of the spectral diffuseness in all retinyl compounds, there are two possibilities for the retinal binding site in rhodopsin. Either binding is quite loose and non-specific at the hydrocarbon end of retinal or, if the cyclohexene ring is bound in a specific orientation, there must be a variety of opsins differing in the torsional angle that they induce onto the chromophore. This would suggest that it might be very interesting to investigate the biological behavior of retinal analogues for which this ring-side chain angle is fixed in various ways. Perhaps someone will have specific information on this point to present at the next workshop.

References

1. Honig, B., Ebrey, T. G.: The structure and spectra of the chromophore of the visual pigments. Biophys. Bioeng. **3**, 151 (1974)
2. Hudson, B., Kohler, B. E.: A low-lying weak transition in the polyene α,ω-diphenyloctatetraene. Chem. Phys. Lett. **14**, 299 (1972)
3. Hudson, B., Kohler, B. E.: Polyene spectroscopy: The lowest energy excited singlet state of diphenyloctatetraene and other linear polyenes. J. Chem. Phys. **59**, 4984 (1973)
4. Christensen, R. L., Kohler, B. E.: Vibronic coupling in polyenes: High resolution optical spectroscopy of 2,10-dimethylundecapentaene. J. Chem. Phys. **63**, 1837 (1975)
5. Christensen, R. L., Kohler, B. E.: High-resolution optical spectroscopy of polyenes related to the visual chromophore. J. Phys. Chem. **80**, 2197 (1976)
6. Hudson, B., Kohler, B. E.: Linear polyene electronic structure and spectroscopy. Ann. Rev. Phys. Chem. **25**, 437 (1974)
7. Strickler, S. J., Berg, R. A.: Relationship between absorption intensity and fluorescence lifetime of molecules. J. Chem. Phys. **37**, 814 (1962)
8. Schulten, K., Karplus, M.: Of the origin of a low-lying forbidden transition in polyenes and related molecules. Chem. Phys. Lett. **14**, 305 (1972)
9. Schulten, K.: Angular momemtum transfer in reactive collisions-correlated excitations in conjugated molecules. Thesis, Harvard University (1974)
10. Christensen, R. L., Kohler, B. E.: Low resolution optical spectroscopy of retinyl polyenes: Low lying electronic levels and spectral broadness. Photochem. Photobiol. **18**, 293 (1973)
11. Sondheimer, F., Ben-Ephriam, D. A., Wolorsky, R.: Unsaturated macrocyclic compounds. VII. The prototropic rearrangement of linear 1,5-enzymes to conjugated polyenes. The synthesis of a series of vinylogs of butadiene. J. Amer. Chem. Soc. **83**, 1675 (1961)
12. For example, Vetter, W., Englert, G., Rigassi, N., Schwieter, U.: In: The carotenoids, Chap. IV. (ed. O. Isler). New York: Halsted Press 1972

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