The Role of Carbonium Ions in Color Reception

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ABSTRACT It is a fundamental property of conjugated systems to accept a proton or Lewis acid and form a stable carbonium ion. Polyenes that are protonated or add Lewis acids in this manner undergo substantial red shifts. For example, vitamin A_1 acetate absorbs at 350 mμ in neutral and at 650 mμ in acidic benzene solution. The fundamental basis for absorption of polyene systems was described in detail in quantum mechanical terms. Applying the carbonium ion treatment to the visual chromophores retinal$_1$ and retinal$_2$ gives a very satisfactory explanation why these polyenes can be made to absorb in the visual region. Furthermore, by proper placement of the Lewis acid several absorption maxima can be gained from the carbonium ions which result. This treatment can be applied to explain experimental results. Individual cones from the frog are now known to absorb at 455, 537, and 625 mμ. If the value for the green cone (537 mμ) is used to calculate the V_o value in Kuhn's equation, the other two wave lengths may then be calculated. The calculated values are 460 and 600 mμ; this is in good agreement with the results from experiment.

To compounds such as retinal and retinylidenealkylamine, the initial role of visual reception has been assigned. Yet, none of these rather simple organic compounds including protonated retinylidenemethylamine has an absorption maximum greater than 440 mμ, and this wave length is just barely in the visible region. However, when the simple organic chromophore is attached to the proper protein, there is a very large red shift in the absorption maximum to values as high as 625 mμ (1). As yet there has been no satisfactory explanation of this shift. Hubbard (2) demonstrated that in cattle rhodopsin (498_1) there is a single prosthetic group consisting of a single molecule of retinal. Therefore any explanation of the red shift must be based on an accepted chemical or physical property that is inherent in a single molecule of chromophore itself. The theory presented here not only attempts to give a chemical and physical basis for the red shift, but also it attempts to explain how a single molecule of retinal can be made to absorb at three discrete wave lengths.

Carbonium Ions and the Red Shift

Recent experiments have shown that polyenes, when placed in a proper acid environment, give rise to carbonium ions that may be examined spectrophotometrically. De Vries (3) isolated cyclic polyenes which contained five conjugated double bonds. Solutions of these polyenes had λ_{max} at such expected wave lengths as 340 to 390 mμ, whereas in the presence of a strong proton or Lewis acid the λ_{max} was shifted to 552 or 602 mμ respectively. De Vries attributed absorption at longer wave lengths to the acid-stabilized carbonium ions. Deno *et al.* (4–6) did considerable work on carbonium ions arising from compounds containing two conjugated double bonds in either cyclic or linear systems. He verified the presence of stable carbonium ions by examining changes in freezing point depression, NMR spectra, and kinetic data as well as shifts in electronic spectra. In general, compounds that normally absorbed near 225 mμ were shifted to values near 300 mμ. Wassermann (7) showed that vitamin A_1 acetate in the presence of a strong acid absorbs at 650 mμ whereas in neutral solution it absorbs at 330 mμ.

Vitamin A_1 acetate, of course, is close in structure to retinal$_1$, and the implications for the chemistry of vision are obvious. To change vitamin A_1 acetate from an ultraviolet absorber to a visible absorber, only the electronic environment need be changed. If vitamin A_1 acetate is allowed to react with a sutiable Lewis or proton acid, it is converted into a carbonium ion and gives a substantial red shift. Carotene with its eleven conjugated double bonds also gives rise to a red shift. In 1954, Wassermann (8) showed that proton acid solutions undergo a shift from 460 mμ to 950 mμ. Shifts in the electronic spectrum of carotene were discussed by Platt (9). Lupinski (10) also showed that the iodine-carotene complex absorbs at 1000 mμ. Carbonium ions from carotene are unusual in that the λ_{max} is shifted from the visible at 460 mμ to the near infrared at 1000 mμ.

From the foregoing evidence, it can be concluded that it is an inherent property of conjugated systems to form stable carbonium ions which absorb at much longer wave lengths than the parent polyenes. Carbonium ions made from polyenes containing from three to seven conjugated double bonds would be expected to have λ_{max} in the visible region. Thus, if retinal reacts with the visual protein to form a carbonium ion it should absorb at the red end of the visible spectrum.

Quantum Mechanical Basis of Light Absorption

The work summarized above shows that the amount of red shift is a function of the length of the conjugated sequence. Kuhn (11) examined the relationship between λ_{max} of the longest absorbing wave length and the structure of the chromophore in various dyes. He recognized two limiting kinds of conju-

gated systems. First there is the symmetrical case which can be written with two extreme resonance structures that make equivalent contributions and in which the π-electrons are considered as a one dimensional free electron gas along the length of the chain. The wave length may be calculated from these considerations

$$\lambda_1 = \frac{8mc}{h} \frac{L^2}{N+1} \qquad (a)$$

and is known as the "isoenergetic wave length." In the second case, that of the unsymmetrical polyene, the free electron treatment no longer applies and the π-electrons are considered to be in a one dimensional potential having a sine wave periodicity of amplitude V_o.

$$\lambda_1 = \left[\frac{V_o}{hc}\left(1 - \frac{1}{N}\right) + \frac{h}{8mc}\frac{N+1}{L^2}\right]^{-1} \qquad (b)$$

The wave length relationship is given in (*b*), which in turn will reduce to (*a*) when V_o assumes a value of zero. For both expressions, m is the electron mass, c the velocity of light, h Planck's constant, N the number of π-electrons, and L the zigzag length of the polymethine chain as defined by Kuhn (11).

Kuhn considered symmetrical polymethine dyes as examples of case one and normal polyenes such as hexatriene as examples of case two. In normal polyenes, V_o assumes values near 2.00 ev. He considers other dyes of an unsymmetrical nature to fall between these two limits, and in these intermediate cases V_o will assume values between zero and 2.00 ev. However, he does not consider the promotion of a polyene to its carbonium ion, although these ions most assuredly could qualify to a first approximation as symmetrical polymethines. Consequently, two wave lengths can be calculated for each polyene, one for the simple polyene and one for the symmetrical polymethine carbonium ion. Obviously depending on the restrictions set by structure, the chromophore may absorb at intermediate wave lengths between these limits.

Trichromatic Absorption by a Single Chromophore

A satisfactory theory of color reception must not only explain how a chromophore can be made to absorb in the visible region, but it must also explain how a single chromophore can be made to absorb at three discrete wave lengths. A successful answer to these problems can be developed from quantitative examination of the carbonium ion theory. Using formula (*a*) the first absorption maximum of 717 mμ can be calculated for vitamin A_1 acetate and compared with the 650 mμ from experiment. To make the calculation, it is assumed that a proton or Lewis acid adds to C_5 or C_{14} (Fig. 1), to produce the most stable carbonium ion. Obviously, from comparison of the

calculated and experimental wave length, it can be concluded that the resulting carbonium ion is not completely symmetrical and V_o has a finite value. If V_o is calculated for the case when λ_{max} is 650 mμ, it in turn may be used to calculate other λ_{max}. For example, assume that in some way a proton or Lewis acid could be made to add to C_{12}; the calculated λ_{max} would be 545 mμ. If a proton or Lewis acid could be made to add to C_{10}, the resulting carbonium ion would absorb at 440 mμ. Thus if a proton or Lewis acid can be placed on a polyene in a stereospecific manner so as to produce carbonium ions of decreasing length, these ions will absorb at 650, 545, and 440 mμ respectively. These are the wave lengths of the three primary colors of light—red, yellow, and blue. Thus nature can solve the problem of trichromatic reception by adding a Lewis acid to three separate loci on the polyene chromophore.

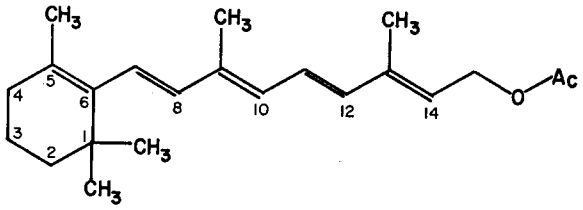

FIGURE 1

Direct Application of Carbonium Ions to Biological Situations

Fig. 2 shows how a chromophore, in this case retinal$_2$, enters into an arrangement with a visual protein in such a manner to produce a carbonium ion. Retinal$_2$ is bound to the protein in the accepted manner by formation of a Schiff base (12). Three separate species are shown, and they would correspond to visual pigments that would selectively absorb red, yellow, or blue light respectively. The specific carbonium ion is made when the protein inserts a Lewis acid at the required position on the retinal$_2$ chain. Note that the acid is not placed on the nitrogen. If it were placed there, the resulting charge would be localized, and a much less symmetrical carbonium ion would be produced that would absorb at shorter wave lengths. In fact even though seven double bonds are involved, this species absorbs at 460 mμ (12).

With the advent of microspectrophotometry the existence of trichromatic receptors has been verified experimentally for a number of species (1, 13–15). And thus it is reasonable to assume that each cone cell manufactures one of at least three different visual proteins. For example, red cones manufacture a specific protein that can react with a retinal in such a manner as to add a Lewis acid to the C_{14} atom. Other cones produce other stereospecific proteins.

Two groups of workers have determined the absorption maxima for in-

dividual goldfish cones by microspectrophotometry (1, 15), and their data can be used to test quantitatively the carbonium ion theory. For calculation purposes, retinal$_2$ is assumed to be the chromophore in goldfish cones since it is found in fresh water fish (12). Calculations involving retinal$_1$ are equally

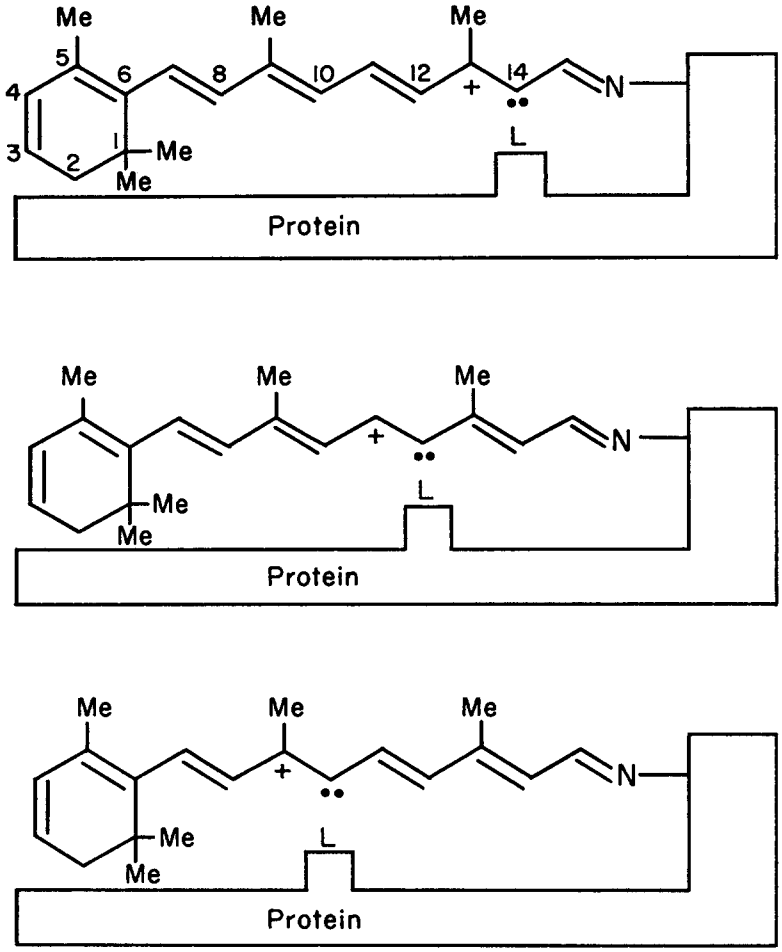

FIGURE 2. This figure shows the protein stereospecifically adding the Lewis acid at the C_{14}, C_{12}, and C_{10} positions respectively. In each case, a carbonium ion is produced that has a shorter electron cloud and therefore absorbs at a shorter wave length.

valid and in good agreement with experiment since only changes in V_o are involved. V_o was calculated from expression (*b*). It was assumed that a Lewis acid added to C_{12} to produce a carbonium ion stabilized by four conjugated double bonds that had an λ_{max} of 537 mμ. When V_o was obtained, the λ_{max} for carbonium ions stabilized with five and three double bonds was calculated, and these values are compared in Table I. For this type of calculation the agreement is excellent.

TABLE I

CALCULATION OF ABSORPTION OF CARBONIUM IONS BASED ON V_o = 10.60 × 10^{-13} ERGS AND COMPARISON WITH λ_{max} FOR GOLDFISH

No. of double bonds in carbonium ion	Calculated λ_{max}	Experimental λ_{max}	
		Liebman	Marks
5	600	625	610
4	—	537	535
3	465	455	460

The carbonium ion theory offers a suitable explanation for the spectrophotometric properties of rhodopsin λ_{max} 500 mμ, porphyropsin λ_{max} 522 mμ, iodopsin λ_{max} 562 mμ, and cyanopsin λ_{max} 620 mμ summarized by Wald (16). Consider that opsin is so constituted that it directs a Lewis acid in a stereospecific manner with reference to the Schiff nitrogen. Rod opsin adds the Lewis acid to C_{12} of retinal, producing a carbonium ion stabilized by three double bonds which absorbs at 500 mμ. When rod opsin adds the Lewis acid to retinal$_2$ the same steric requirements must be met, and a carbonium ion with four double bonds—an extra one in the ring at the 3,4 position—is generated that absorbs at longer wave lengths. Cone opsin apparently adds the Lewis acid stereospecifically to C_{14}. With retinal$_1$ there are four double bonds in the carbonium ion and with retinal$_2$ there are five. Thus the arrangement of λ_{max} from the resulting combinations is completely predictable.

Consideration of the Biological Lewis Acid

It has been shown that opsin contains two thiol groups as well as a proton-liberating group that is thought to be imidazolonium (17, 18). Fig. 3 is merely a modification and extension of the scheme already proposed by Hubbard and Kropf (19) and Wald *et al.* (16). The chromophore is fastened to the protein covalently by a Schiff base linkage, which is probably the only covalent linkage, and itself plays no part in the spectral properties. According to the above authors, the chromophore fits into a pocket in the protein. This pocket

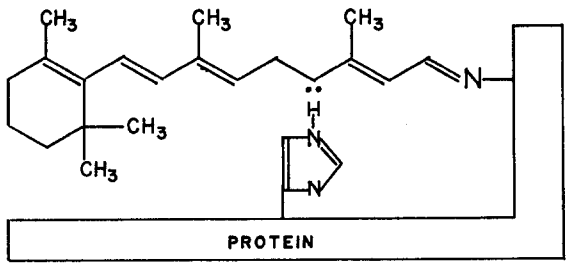

FIGURE 3

probably confines the chromophore in a preferred spatial arrangement, and with the chromophore fixed precisely, the imidazolonium ion may now impinge upon a certain and specific carbon atom. In Fig. 2, the imidazolonium ion is shown impinging on the C_{12} position. As the positive charge approaches the polyene at the desired locus, the polyene is converted into a carbonium ion.

Kropf and Hubbard (20) have proposed a hypothesis to explain the red shift in retinal chromophores. They propose that the imino nitrogen of the Schiff base is first protonated. They next propose that negatively charged groups are in some way associated with the chromophore in such a manner as to smear out the electron cloud and in this way lower the excitation energy. The hypothesis involving participation of both a proton and negative groups is not given in detail and is not based on any given chemical model systems. Consequently it is difficult to describe in quantitative terms. Furthermore, it is difficult to transpose the implied mechanism to the field of color differentiation since it does not explain either qualitatively or quantitatively how a single chromophore can absorb at three separate wave lengths.

Wald (21) has shown that metarhodopsin I is converted to metarhodopsin II almost immediately at 5°C in the dark. Apparently he finds no spectrophotometric evidence for the existence of other intermediates. In this transformation, the λ_{max} shifts from 478 mμ to 380 mμ with no intermediate value of 440 mμ—the λ_{max} for the imino compound. If the negative group were pulled away from the chromophore, the λ_{max} would be expected to shift from 478 mμ to 440 mμ. On the other hand at physiological pH it is difficult to see why the proton should be removed either as a first or second step in the sequence.

There appears to be no experimental evidence for the existence of protonated Schiff's nitrogen in visual pigments. Additionally, the mechanism involves the cooperation of two oppositely charged groups in such a way as to produce a more uniformly distributed electron cloud. Apparently systems of this kind are not known to organic chemistry. Furthermore, the same result is obtained in a much simpler and chemically straight forward fashion by forming either a carbonium ion, carbanion, or free radical. Examples of these systems are known in great detail both experimentally and theoretically. For these reasons the above mentioned mechanism is of doubtful status.

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