Structures of the Visual Chromophores and Related Pigments: A Conformational Basis of Visual Excitation

(11-cis retinal/vitamin A/xanthenoid/crystal structure/carotenoid)

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ABSTRACT A detailed conformational analysis of the known crystal structures of vitamin A, carotenoid, and xanthenoid derivatives has been made. These studies suggest that the visual chromophore 11-cis retinal and its isomers may each occur in several different conformational states, which would be expected to have different light-absorbing characteristics. The presence of such states for 11-cis retinal and all-trans retinal may contribute to the observed rhodopsin intermediates that have been characterized by different λmax values, and to the breadth of spectral response in scotopic and color vision.

In the process of scotopic vision, absorption of light by the visual pigment rhodopsin gives rise to nerve transduction and the physiological process of vision. Rhodopsin is believed to be integrated into the disc membrane of retinal-rod outer segments as a protein–lipid complex (1). It has been known for many years that the absorption of light by the rhodopsin visual chromophore, retinal, results in the geometrical isomerization of the 11-cis to the all-trans form (2). It has also been shown that the chromophore is linked covalently to the lipoprotein (3, 4), and that it may possibly undergo a change in covalent bonding (5). The mechanism by which the nerve impulse is initiated is still obscure; an early receptor potential accompanying retinal illumination has been observed (6); it may be related to isomerization or to the transfer (5) of the chromophore, but it is not clear whether this potential is part of the mechanism of excitation.

While both the chromophore and the lipoprotein are essential components in the primary processes of vision, little is known of their conformations in rhodopsin. A knowledge of the detailed conformation of rhodopsin and of its constituents will, however, be essential for a precise understanding of the photochemical events. In the present paper, we give a conformational analysis of the visual chromophores and discuss its relevance to the visual process. In addition, using information from crystallographic studies on the interactions of small molecules with proteins, we suggest models for the interaction of the chromophore with opsin in rhodopsin.

Structural analysis

Crystal structures of the visual chromophores 11-cis retinal and all-trans retinal (7), and of eight other carotenoid and xanthenoid derivatives are available (8–15). The chemical structures and atomic numbering of these compounds are given in Fig. 1. Detailed coordinates have been published for the first six of these compounds, while for compounds 7 and 8, only preliminary reports have been made and the coordinates are not yet available.

The geometry around the double bonds in the carotenoid chains can be either cis or trans. Therefore, several geometrical isomers are possible (16). In addition, the conformation about the ring-chain 6-7 linkage can be gauche (‘s-cis’) or anti (‘s-trans’), and each geometrical isomer can potentially exhibit different conformations for the ring and ‘single’ bonds in the side chain.

Torsion Angles. The torsion angles in the known compounds were calculated from published atomic coordinates; they are listed in Tables 1 and 2.

Ring Conformation. The torsion angles about the ring bonds for the compounds are listed in Table 1. All of the cyclohexene rings are puckered in the half-chair conformation. The atoms C(2) and C(3) are displaced (about 0.3 Å) on opposite sides of the plane through the remaining four atoms. The largest torsion angle is about the C(2)–C(3) bond, which is opposite the double bond, while the smallest torsion angle is about the double bond. In the case of compounds 5 and 6, the keto group at position C(4) influences the conformation of the cyclohexene ring such that the torsion angles about 2-3 and 3-4 are, respectively, reduced by about 10° and 20° relative to those of the β-ionylidene ring; the former becomes about equal to the torsion angle about the 1-2 bond. Therefore, in general, the cyclohexene rings are less puckered than the cyclohexene rings. In vitamin A1 (retinal 2) there is an additional double bond at position 3-4 in comparison with vitamin A1 (retinal 1). It is expected that the cyclohexadiene ring system will preferably adopt a conformation similar to that of the cyclohexene ring, or more planar. The substituent C(16) lies close to the ring plane, whereas one of the gem dimethyl groups on C(1) displays a pseudo-axial orientation, while the other displays a pseudo-equatorial orientation.

Ring-chain conformation. The stereochemistry of the ring relative to the chain is an important parameter in the retinals and the carotenoid derivatives. The ring-chain conformation is described by the torsion angle χ [5-6-7-8] or 1-6-7-8, which is the angle between the ring double-bond 5-6 and the chain double-bond 7-8 (Table 2). The usual usage of the terms trans and cis denotes the configuration around double bonds. Where these terms are applied to geometries around single bonds, they are written ‘s-trans’ and ‘s-cis’, respectively. However, the observed torsion angles cannot be adequately described in this way. Therefore, we shall use the conventional terminology for single bonds, anti and gauche. Rotation about the ‘single’ bond 6-7 is not free; instead, it is highly restricted...
Fig. 1. The chemical structures and numbering of the known crystal structures of vitamin A, carotenoid, and xantheneoid derivatives.
1 trans-β-ionylidene-γ-crotonic acid (8)
2 Vitamin A acid (9)
3 β-Carotene (10)
4 15,15'-Dehydro-β-carotene (11)
5 Canthaxanthine (12)
6 15,15'-dehyrocanthaxanthine (15)
7 cis-β-ionylidene-γ-crotonic acid (13)
8 retro-β-ionylidene acetyl-p-bromoanilide (14)
9 11-cis retinal (7)
10 All-trans retinal (7)

Fig. 2. A diagrammatic illustration of possible conformations for the cis-and trans-isomer of the retinal chromophore in rhodopsin. The chromophore is shown bound to a lysine residue ('Model 2').
A The Schiff base can exist as the syn or anti isomer. It is conceivable that isomerization of the Schiff base may accompany isomerization at the 11-12 bond.
B The presence of the 6-7 anti conformer of 11-cis retinal (shown) might be expected to enhance the red shift.
C An induced conformational change in opsins, such as that shown figuratively for Cα of the lysine residue, might lead to initiation of the nervous impulse.
to three possible ranges. In the anti conformation, the \( \chi \) angle is about 180°, while in the gauche conformations, the angle is in the range 39 to 52° (+gauche) or −39 to −52° (−gauche). Compound 1 is the only example that exhibits the anti conformation, \( \chi = 169° \). The cis planar conformation about the 6-7 bond is forbidden because of steric interaction between the substituents in the ring positions C(5) and C(1) and the hydrogen atoms in the side-chain at C(8) and C(7). Therefore, although the anti planar conformation is allowed, the +gauche conformation is preferred (17). Both 11-cis and all-trans retinals exhibit the gauche conformation (7).

**Chain Conformation.** Here one has to consider two possibilities, an all-trans chain and one containing cis linkages. In the known trans compounds, the torsion angles about the double and ‘single’ bonds in the side chain lie, on an average, in the range 180 ± 5°, emphasizing the fact that small distortions from planarity are often encountered, even in conjugated double-bonded systems. In general, the chain displays a bend normal to its plane (15). In compound 7 there is only a small deviation from the cis planar arrangement, perhaps due to steric interaction between the hydrogen atoms attached to positions C(8) and C(11). Similarly, small distortions from anti planarity are observed in the retinals (7). In vitamin A acid (2), the torsion angle 13-14-15-0 involving the carbonyl group is 16°, while that involving the hydroxyl group 13-14-15-OH is 171°. However, in 11-cis retinal and all-trans retinal, the torsion angle 13-14-15-O for the carbonyl group is about 180°. For the Schiff base complex between retinal and opsin, either of the corresponding conformations, or an intermediate one, might be present.

**Discussion.**

**Conformations of the Chromophore.** In the absence of molecular crowding, one would expect that the conformations

**TABLE 1. Torsion angles about the cyclohexene ring bonds**

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \phi_1 )</th>
<th>( \phi_2 )</th>
<th>( \phi_3 )</th>
<th>( \phi_4 )</th>
<th>( \phi_5 )</th>
<th>( \phi_6 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-( \beta )-ionylideneg-crotonic acid</td>
<td>±44</td>
<td>±60</td>
<td>±41</td>
<td>±11</td>
<td>±3</td>
<td>±12</td>
</tr>
<tr>
<td>Vitamin A acid</td>
<td>±39</td>
<td>±59</td>
<td>±45</td>
<td>±14</td>
<td>±8</td>
<td>±7</td>
</tr>
<tr>
<td>( \beta )-Carotene</td>
<td>±21</td>
<td>±35</td>
<td>±26</td>
<td>±4</td>
<td>±9</td>
<td>±1</td>
</tr>
<tr>
<td>15,15'-Dehydro-( \beta )-carotene</td>
<td>±42</td>
<td>±60</td>
<td>±44</td>
<td>±12</td>
<td>±6</td>
<td>±9</td>
</tr>
<tr>
<td>Canthaxanthine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residue 1</td>
<td>±50</td>
<td>±49</td>
<td>±23</td>
<td>±0</td>
<td>±4</td>
<td>±28</td>
</tr>
<tr>
<td>Residue 2</td>
<td>±45</td>
<td>±40</td>
<td>±15</td>
<td>±6</td>
<td>±2</td>
<td>±27</td>
</tr>
<tr>
<td>15,15'-Dehydrocantha-xanthine</td>
<td>±49</td>
<td>±51</td>
<td>±27</td>
<td>±2</td>
<td>±1</td>
<td>±24</td>
</tr>
</tbody>
</table>

The torsion angle (\( \phi_{12} \)) about a bond is defined as the angle made by the projection of the 1-2 bond with respect to the 3-4 bond. When viewed down the 2-3 bond, positive angles (0 to +180°) are for a clockwise rotation of the far bond 3-4 relative to the near bond 1-2, while negative angles (0 to −180°) are for a counterclockwise rotation. Zero angle is defined for an eclipsed position of the 2-3 and 3-4 bonds. The upper and lower signs for the torsion angles indicate the conformations of the enantiomers. Since all of the compounds belong to a centrosymmetric space group, both enantiomers are present in the crystal. Although the torsion angles are not available for compound 7 and the retinals, the retinals exhibit the preferred half-chair conformation.

**Conformational Basis of Visual Excitation**

**TABLE 2. The ring-chain torsion angles**

<table>
<thead>
<tr>
<th>Compound</th>
<th>5-6-7-8</th>
<th>1-6-7-8</th>
<th>Conformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-( \beta )-ionylideneg-crotonic acid</td>
<td>+196°</td>
<td>±13°</td>
<td>anti</td>
</tr>
<tr>
<td>2 Vitamin A acid</td>
<td>±48°</td>
<td>±143°</td>
<td>±4°</td>
</tr>
<tr>
<td>3 ( \beta )-Carotene</td>
<td>±39°</td>
<td>±143°</td>
<td>±4°</td>
</tr>
<tr>
<td>4 15,15'-Dehydro-( \beta )-carotene</td>
<td>±49°</td>
<td>±132°</td>
<td>±4°</td>
</tr>
<tr>
<td>5 Canthaxanthine</td>
<td>±52°</td>
<td>±127°</td>
<td>±4°</td>
</tr>
<tr>
<td>Residue 1</td>
<td>±52°</td>
<td>±127°</td>
<td>±4°</td>
</tr>
<tr>
<td>Residue 2</td>
<td>±52°</td>
<td>±127°</td>
<td>±4°</td>
</tr>
<tr>
<td>6 15,15'-Dehydrocantha-xanthine</td>
<td>±43°</td>
<td>±141°</td>
<td>±4°</td>
</tr>
<tr>
<td>cis-( \beta )-ionylideneg-crotonic acid</td>
<td>80°</td>
<td>—</td>
<td>gauche</td>
</tr>
<tr>
<td>8 retro-( \beta )-ionylideneg-acetyl-p-bromoanilide</td>
<td>180°</td>
<td>—</td>
<td>anti</td>
</tr>
<tr>
<td>9 11-cis retinal</td>
<td>±40°</td>
<td>—</td>
<td>±4°</td>
</tr>
<tr>
<td>10 All-trans retinal</td>
<td>±59°</td>
<td>—</td>
<td>±4°</td>
</tr>
</tbody>
</table>

Since all of these crystal structures possess a center of inversion, both enantiomers are present. The upper and lower signs in the torsion angles designate the enantiomeric conformations. The detailed structures of compounds 7, 8, 9, and 10 have not appeared; only approximate values of the torsion angles 5-6-7-8 are available. It is noteworthy that compound 8 has a double bond linking the ring to the chain and, therefore, it is not strictly analogous to the other compounds.

about single bonds in a conjugated system will be planar, to maximize conjugation. It has long been recognized that in 11-cis retinal there is molecular overcrowding, involving the methyl group at position 13 and the hydrogen atom at position 10, but it was not clear whether the steric interactions are relieved by rotation about the cis double bond, the adjacent single bonds, or both (16). In the recently determined x-ray structure of 11-cis retinal, steric interaction was relieved by rotation about the 12-13 bond; the conformation about the 10-11 bond was close to antiplanar (7). Honig and Karplus (18) have also predicted a similar conformation from calculations of the torsional potentials of retinal for some conformations about the 10-11, 11-12, and 12-13 bonds. It is interesting to compare this situation with systems containing isolated double bonds. An analysis of the conformations about isolated cis and trans double bonds demonstrated that the single bonds adjacent to the double bond show considerable "flexibility" to rotation, and that equal rotations of opposite sign (125° ± 20°) about these bonds are preferred (19).

However, in conjugated systems the relative rotations about single bonds appear to be those that favor conjugation. Rotation about the 10-11 bond will decrease the degree of conjugation to a larger extent than rotation about the 12-13 bond, and a smaller rotation about the 10-11 bond would be expected for the ground state. Hence, conjugation, in part, differentiates the flexibilities of the single bonds. It should, however, be noted that the presence of a bulkier substituent at position 13 than at position 10 in retinal might also lead to unequal rotations about the single bonds.

In its complex with opsln, the visual chromophore may not exist in the identical conformation as in free solution or in a crystal. Due to the possibility of 'flexibility' about the single bonds, a range of conformations are possible that differ only slightly in energy. Indeed, Honig and Karplus (18) have
found the presence of two energy minima corresponding to two conformations about the 12-13 bond, one about 60° and the other about 130°. From energy calculations (18), it appears that the energy difference between these two conformations is small and that the entire range of conformations 60°–130° about the 12-13 bond can be exhibited by the retinals. The presence of different conformers in rhodopsin would be expected to broaden the absorption spectrum, since individual conformers will possess different degrees of conjugation and, hence, different spectral characteristics. However, this mechanism alone does not explain the red shift observed for rhodopsin, relative to retinal (20).

**A Conformational Model for Rhodopsin.** In the absence of detailed structural information concerning the conformation of the chromophore and its environment in rhodopsin, one might use the present knowledge of possible conformations for the chromophore, and of the types of interactions that have been observed between ligands and proteins in x-ray crystallographic studies, to construct a model for the active site of rhodopsin. In view of the markedly different $\lambda_{\text{max}}$ values for rhodopsin and for the free chromophore in homopolar solvents (20), one may suggest that while the chromophore is likely to interact in similar ways with ligands of comparable hydrophobicity, the presence of specific polar groups in suitable positions in opsin may be responsible for the red shift. As models for the interaction of the cyclohexene ring in rhodopsin, one might consider the interaction of the heme group in myoglobin (21), hemoglobin (22), and ferricytochrome c (23), of the tyrosyl side chain of glycyrl-tyrosine in its complex with carboxypeptidase A (24), and of the indolyl side chain of formyl-tyrosylphepso in its complex with alpha-chymotripsin (25). In all of these examples, the hydrophobic group binds in a pocket in the protein, with the formation of many van der Waals contacts; similarly, we imagine that the cyclohexene ring of retinal is enclosed in a pocket in the opsin within which it makes favorable contacts. To elaborate the model further, we must consider whether the chromophore undergoes a change of covalency. It has been suggested that the chromophore undergoes a change in covalent bonding in a dark process after illumination (5). Retinal is linked to phosphatidyl ethanolamine in dark rhodopsin, and in the illumination product metarhodopsin I, but to have become transferred to a lysine residue of the opsin in metarhodopsin II. However, it has since been suggested that the observed linkage of the chromophore to phospholipid is induced by the analysis and isolation procedures (26). We will, therefore, consider both the case where the chromophore is transferred from phospholipid (*Model 1*) and the case where it is covalently linked only to lysine (*Model 2*).

**Model 1.** If we assume that in rhodopsin, metarhodopsin I, and metarhodopsin II the cyclohexene ring remains bound at the same site, then evidently conformational changes in the chromophore are likely during the transfer of the Schiff base linkage from the primary amine of phosphatidyl ethanolamine to that of lysine. These conformational changes are additional to those induced directly by the absorption of light quanta. In the model we describe here, we suggest that the isomerization induced by absorption of light will induce a strain in the chromophore, in view of its attachment at each end to protein and phospholipid, and that the strain may be relieved by a conformational change of the phospholipid and/or protein.

Since these components constitute an integral part of the disc membrane of the rod outer segment, such changes may initiate ion passage through the membrane and, thus, initiate the process of nerve transduction. We further suggest that the movement of the Schiff base brings it close to a lysyl side chain, which displaces the phospholipid from the chromophore. The phospholipid is then free to move to its original position in the membrane, hence stopping the local induced ion flow and terminating the pulse of current. It is this current that may correspond to the early receptor potential. A feature of this theory is that a phospholipid molecule is strongly bound through the chromophore to the protein in unbleached, but not in bleached, rhodopsin. Significantly, more phospholipid can be extracted from rod segments after photo-bleaching (27). The visual cycle is completed by the reverse isomerization of the chromophore, and its transfer to the phospholipid.

**Model 2.** If we assume that the chromophore does not change covalent linkage but that, as in *Model 1*, the cyclohexene ring remains bound at the same site in the lipoprotein during the 11-cis to all-trans isomerization, we must consider whether there is sufficient flexibility in the side chain of the lysyl residue and in the chromophore for the isomerization to occur without inducing further large conformational changes in the opsin. In Fig. 2 we show schematically possible conformations for the 11-cis and all-trans isomers of the chromophore linked to the lysyl residue. It is evident that there could be sufficient flexibility of the lysyl side chain and the chromophore side chain beyond the 11-12 double bond to permit the isomerization. The reverse isomerization may occur spontaneously to relieve the strain in the rhodopsin, or may occur after hydrolysis of the chromophore from the opsin.

In both of these models, the 11-cis → all-trans isomerization of the chromophore is considered to involve conformational changes primarily distal to the 10-11 bond in the chromophore, as shown in the crystal structure of the 11-cis isomer (7). These changes are considered responsible for the metarhodopsin I→metarhodopsin II transition. Metarhodopsin I ($\lambda_{\text{max}} = 478$ nm) shows a red shift relative to metarhodopsin II ($\lambda_{\text{max}} = 380$ nm) and all-trans retinal ($\lambda_{\text{max}} = 387$ nm) (28); this shift may arise from the nature of the local environment at the distal end of the 11-cis chromophore. It has been suggested that the chromophore may exist in rhodopsin as a protonated Schiff base (29); recently, it was shown that the retinylideneiminium chromophore can show a red shift similar to that of rhodopsin (30). A large bathochromic shift was observed for the retinylideneiminium chromophore at low temperature in the presence of trichloroacetic acid, but not in the presence of hydrochloric acid, acetic acid, or monochloroacetic acid; it was suggested that a specific association of trichloroacetic acid with the retinylideneiminium chromophore might be responsible (30). Such an association is likely to be dependent on the conformation of the retinylideneiminium chromophore, and the interaction with trichloroacetic acid may be associated with the occurrence of a specific conformation of the chromophore at low temperature. In its complex with opsin, the red shift observed for rhodopsin and metarhodopsin I may be due to specific interactions between the 11-cis-chromophore and the opsin that are lost when the chromophore isomerises to all-trans. It has been suggested that the red shift may arise from delocalization of
the positive charge of the protonated Schiff base to the side-chain carbon atoms of the chromophore (28); alternatively, it has been suggested that the Schiff base is uncharged, but that carbonyl ions are induced in the side chain by appropriately placed Lewis acids or protonating groups of rhodopsin (31). It is interesting to compare this situation with that proposed for lysozyme from crystallographic studies (32). In lysozyme, it appears that the glycosyl oxygen atom of the substrate is protonated by glutamic acid residue 35, and the formation of a carboxonium ion at C1—O3 is promoted by distortion of the sugar residue towards a half-chair conformation and by the proximity of an anionic carboxylate group, aspartate 52. By analogy with Fig. 2, the cis chromophore in rhodopsin or metarhodopsin I is shown protonated by an acidic group, while the delocalized positive charge is stabilized on carbon atoms at the end of the chromophore side-chain by an adjacent negative group (or dipole). Isomerization to the all-trans isomer positions the distal end of chromophore in a different local environment, which does not promote carbonium ion formation, and the red shift is lost. The spectral difference between rhodopsin and metarhodopsin I may reflect small differences in conformation of the 11-cis chromophore that position it in slightly different environments in the opsin.

We therefore suggest that some of the illumination products of rhodopsin with different \( \lambda_{\text{max}} \) values are characterized primarily by different chromophore conformations, rather than by different opsin conformations, as has been postulated (33). Changes in chromophore conformation could also account for the unmasking of certain functional groups in the rhodopsin that have been observed to accompany the transition between the illumination products (33).

It should be possible to perform structure determinations and a conformational analysis similar to that described here both for the retinylideneiminium compounds and for the so-called retinals (vitamin A \( \lambda \)) chromophore (15). The additional ring double-bond in retinals would be expected to contribute to a greater red shift than observed for the retinals themselves, as would also the occurrence of the 6-7 's-trans' (anti) conformer. Different visual pigments, arising from combinations of the retinal chromophores with different opsins, may possibly be involved in color vision.

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