Polyenes and vision

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Polyenes are important for vision in all sighted species. The visual pigments (the rhodopsins) all use 11-*cis*-retinal as the chromophore; some possible reasons for the importance of this isomer are now emerging. New results on the involvement of xanthophils in the maintenance of the retina are also discussed.

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Introduction

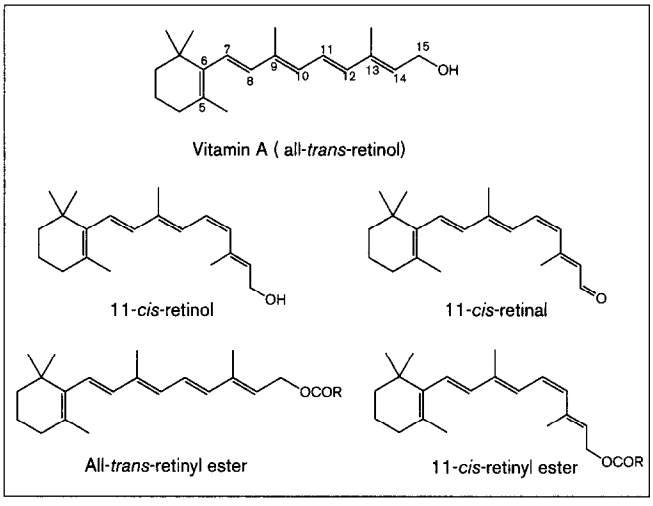
Polyenes are central to vision; the polyene 11-*cis*-retinal is used as the chromophore in all sighted species [1]. What is it about the structure of this molecule that makes it so special? As the molecular events in visual transduction and adaptation are increasingly well understood, we are beginning to realize why the structure of 11-*cis*-retinal is extremely well adapted for the purpose of vision. For example, the structural change on isomerization of 11-*cis*-retinal to all-*trans* retinal is large, and initiates a substantial conformational change in the rhodopsin protein, allowing signal transduction to occur. The size of the required conformational change in the protein also limits thermal isomerization of 11-*cis*-retinal, which would decrease the sensitivity of the system. Equally important, the thermodynamic instability of 11-*cis*-retinal means that it is almost never formed spontaneously, which has important consequences for light adaptation.

Although 11-*cis*-retinal is perhaps the best known polyene component of the visual process, it is not the only polyene involved in vision. A second group of visual polyenes, those of the carotenoid class, has recently attracted a great deal of attention [2]. Foods rich in the xanthophils (lutein and the zeaxanthins) may prevent the degeneration of the macula (the 'yellow spot', which is primarily responsible for high-definition vision) [2], raising the possibility that these compounds may be important in the function or maintenance of this region of the retina. Indeed, it is the concentration of lutein and the zeaxanthins in this region of the retina that gives the macula its characteristic yellow color [3]. Macula degeneration is the major cause of blindness in the elderly, and it is therefore possible that understanding the functions of these molecules, and the structural specificity in their functions, may lead to a novel class of neuronal protecting agents that would be medically important.

Visual signaling

Vision is initiated when a photon is absorbed by rhodopsin, an integral membrane protein found in the highly specialized photoreceptors of the retina [1]. Rhodopsin is taken as the structural and functional paradigm for the seven-transmembrane helical receptors. Much of what has been learned about rhodopsin appears to be applicable to other members of this class of receptors, which includes medically important families such as β-adrenergic, leukotriene, histaminergic, and muscarinic cholinergic receptors [4]. Studies on the rhodopsin signaling system have advanced so far for several reasons including its historical and current importance, the ready availability of large quantities of the

Figure 1



Structures of vitamin A and related retinoids. 11-*cis*-retinal is the active chromophore in rhodopsin.

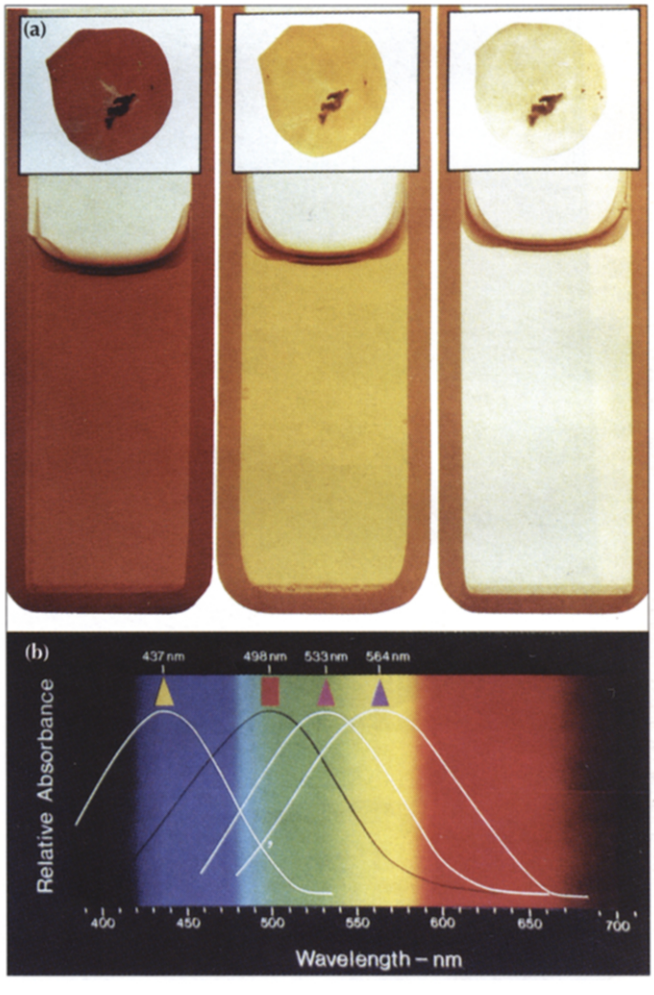
molecular components, and the fact that the molecule has a built-in spectroscopic reporter group (the chromophore), enabling conformational and protonation states to be readily defined.

11-*cis*-retinal (Fig. 1) is the chromophore for rhodopsin, and is responsible for its ability to absorb visible light. The chromophore is covalently linked to the protein via a protonated Schiff base to an active-site lysine residue [2]. The maximum absorption of rod rhodopsin in terrestrial vertebrates is centered at ~500 nm, which coincides with the maximal light emission during the day (Fig. 2). This absorption maximum is shifted in the cone rhodopsins, which are responsible for color vision. These have λ_{max} values of 437 nm (blue), 533 nm (green), and 564 nm (red) in humans (Fig. 2) [5]. The cone rhodopsins contain the same 11-*cis*-retinal chromophore as rod rhodopsin, and so the interactions between the protein and the chromophore must be different in the various proteins, giving rise to the different absorption spectra observed.

As rod rhodopsin absorbs light in the blue-green region of the spectrum, it appears red in solution (Fig. 2). This red color bleaches on exposure to light (Fig. 2), because the 11-*cis*-retinal (in its protonated Schiff base form) isomerizes to the all-*trans* congener, which is eventually hydrolyzed to form the free protein (opsin) and all-*trans*-retinal (Fig. 3a) [1]. After rhodopsin absorbs a photon of light the retinal photoisomerizes within 200 fs to form bathorhodopsin [6], an early and short-lived intermediate that is ~35 kcal mol^{-1} higher in energy than rhodopsin. Since a mole of photons carries 57 kcal mol^{-1} with it, bathorhodopsin captures an extraordinary ~61 % of the light energy. This energy is released thermally as bathorhodopsin decays to the remaining intermediates in the cascade (Fig. 3b).

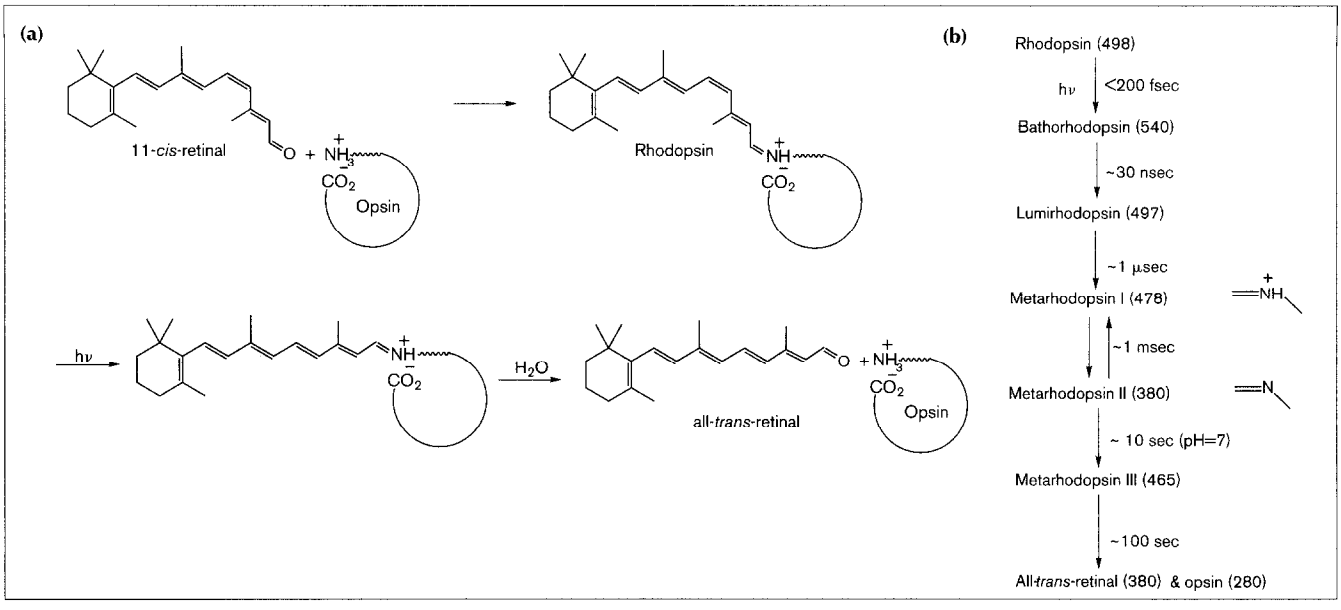
The intermediate states that form in the process of bleaching can be observed spectroscopically (Fig. 3b), and are thought to represent different conformations of the protein. Metarhodopsin II is a particularly noteworthy intermediate, because it is this conformation of the protein that transfers the information that a photon has been absorbed to the heterotrimeric G protein transducin. This stimulates transducin to catalyze the exchange of GTP for GDP [7], activating a phosphodiesterase enzyme that is specific for cyclic GMP [7]. The hydrolysis of cGMP triggers the closing of specific ion channels, causing the hyperpolarization of photoreceptor membranes and initiating the neuronal signal that leads to the visual response (Fig. 4) [7]. Metarhodopsin II is also interesting because it is the only

Figure 2



Bleaching and spectral range of rhodopsin. **(a)** Bullfrog retinas (top) and extracts of bullfrog retinas (bottom). The red color of rod rhodopsin (left) is bleached by exposure to light as rhodopsin is first converted to all-*trans*-retinal and opsin (middle) and finally to vitamin A (all-*trans*-retinol) and opsin (right). **(b)** Difference spectra of the three cone pigments (white lines) and the rod pigment (black line). The colored symbols above represent the color of the pigments. Reprinted, with permission, from [4].

Figure 3



The chemical transformations accompanying vision. **(a)** The chromophore 11-*cis*-retinal is ligated to the apoprotein opsin via a Schiff base yielding rhodopsin. Light causes a *cis* to *trans* isomerization of the retinal that initiates vision; the all-*trans*-retinal is then cleaved from the protein hydrolytically. **(b)** There are many conformational states of rhodopsin that are distinguishable spectroscopically. Light induces an extremely rapid isomerization of the 11-*cis*-retinal to yield bathorhodopsin, which more slowly undergoes conformational changes to give metarhodopsin II, which has an unprotonated Schiff base and activates the G protein transducin. After conversion to metarhodopsin III, all-*trans*-retinal is cleaved from the protein, regenerating opsin.

bleaching intermediate known to possess an unprotonated Schiff base. Deprotonation of this Schiff base is required for the activation pathway. This can be shown by singly methylating the active-site lysine of rhodopsin, and showing that the photobleaching of the methylated pigment can neither form metarhodopsin II nor activate transducin [8].

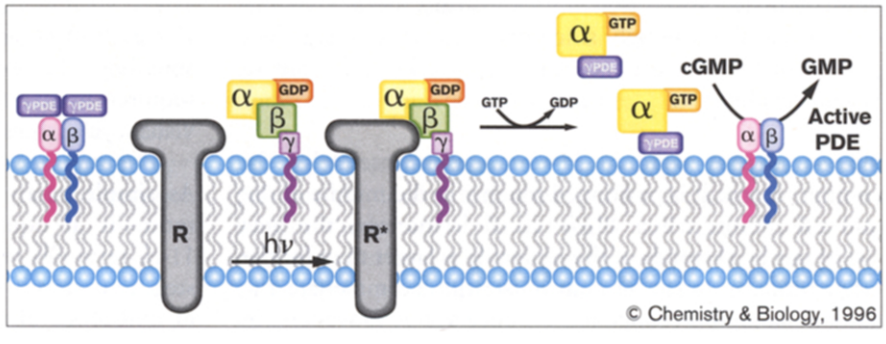
Important features of rhodopsin

Vision is characterized by a remarkably low level of 'dark noise' [9]. Although rhodopsin photoisomerizes in femtoseconds, its rate of thermal isomerization is extremely low, of the order of 1 isomerization in 470 years [9]. Therefore, the signal to noise ratio for rhodopsin (activation in the light versus dark) is an enormous factor of ~10^{23}. This is crucial for effective visual signaling, because rhodopsin is found in extremely high concentrations in rod photoreceptor membranes (in the millimolar range). Significant levels of thermal isomerization of rhodopsin would therefore drastically decrease our ability to perceive dim light. If the human eye is to detect light at the single photon level (which it does), the rate of thermal isomerization of rhodopsin must be extremely low. Indeed, the activation energy for the thermal isomerization of rhodopsin is ~45 kcal mol^{-1} [10].This large activation energy is, at least in part, a consequence of the structure of the chromophore.

The human retina is an enormously sophisticated device that can measure contrast in light over a range of ~10^{10}

Figure 4

Activation of the visual response. Light transforms the light receptor rhodopsin (R) to an activated form (R*) that binds the trimeric G protein transducin. R* promotes the exchange of GDP for GTP on transducin, and two activated α-subunits of transducin (now with bound GTP) bind the two inhibitory γ-subunits of cyclic GMP phosphodiesterase (γPDE), leaving the α- and β-subunits to catalyze the hydrolysis of cGMP. The lower levels of cGMP lead to the closure of cGMP-gated ion channels and the hyperpolarization of the cell.



in background illumination. One of the mechanisms that is important in adaptation is photochemical adaptation. At high light intensities, much of the rhodopsin in the photoreceptors is bleached, decreasing the light-gathering capabilities of the retina and allowing the retina to adapt to the greater light fluxes. The need for adaptation is presumably the reason why bleaching occurs in vertebrates.

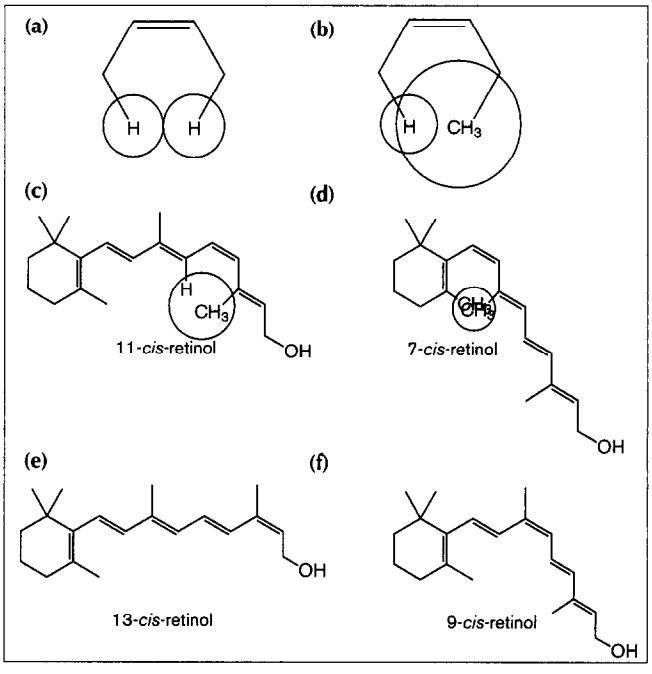
The ability of various opsins to perturb the absorption spectra of the chromophore is a final remarkable aspect of visual pigment function that may well be attributable to the special properties of 11-*cis*-retinal. Since rod rhodopsin absorbs light at ~500 nm, while 11-*cis*-retinal itself has an absorption maximum of 360 nm, a substantial red shift occurs when the chromophore combines with the opsin. Much of this shift is due to the formation of a protonated Schiff base, which in itself shifts the λ_{max} of retinal from 360 to 480 nm [11]. The remaining shifts are specific for different opsins and are referred to as opsin shifts. These require specific interactions between the protein backbone of the opsin and the protonated Schiff base of the chromophore [11,12]. In the case of the visual pigments, the absorption maxima can vary from ~350 nm, for some insect pigments, to 625 nm for the red cone opsin.

Special features of 11-*cis*-retinal

Before commenting on why 11-*cis*-retinal may have been selected as the visual chromophore, it is worth considering the chemistry of this polyene. The vitamin A aldehydes exist as a series of double bond isomers (Fig. 1). Of the known mono-*cis* isomers of retinal, 11-*cis* and 7-*cis*-retinal are the least stable, due to eclipsing interactions (Fig. 5). The position of the *cis* double bond in 11-*cis*-retinal brings the 13-methyl group and the C10 hydrogen atom into close proximity, causing the molecule to twist about its single bonds to avoid closer than van der Waals interactions between these groups. Indeed, 11-*cis*-retinal is 4.1 kcal mol^{-1} higher in energy than all-*trans*-retinal, and is the least stable of the commonly known retinals [13]. In an equilibrium mixture of retinals, only 0.1 % exists as 11-*cis*-retinal. Thus, when rhodopsin is illuminated with visible light, the isomerization reaction in the retinals induced by the absorption of photons runs thermodynamically downhill (although, since the amount of energy in a photon of light is so large, this may not be important in visual signaling).

The *cis* double bond in 11-*cis*-retinal is interesting for another reason. It is positioned roughly in the middle of the molecule; thus, when 11-*cis*-retinal isomerizes, the area swept out by the ends of the molecule is larger than for the other retinals. As the protein must still accommodate the chromophore, this change must induce a large movement in the protein.

Figure 5



The effect of eclipsing interactions on the stability of retinoid isomers. **(a)** The close approach possible for two hydrogen atoms is prevented by the larger atomic radius of a methyl group in a similar molecule **(b)**. Interactions between an H and a CH_3, or two CH_3 groups, strain the structure of 11-*cis*-retinoids **(c)** and 7-*cis* retinoids **(d)**, but not 13-*cis*-retinoids **(e)** or 9-*cis*-retinoids **(f)**.

Energetics of 11-*cis*-retinal function

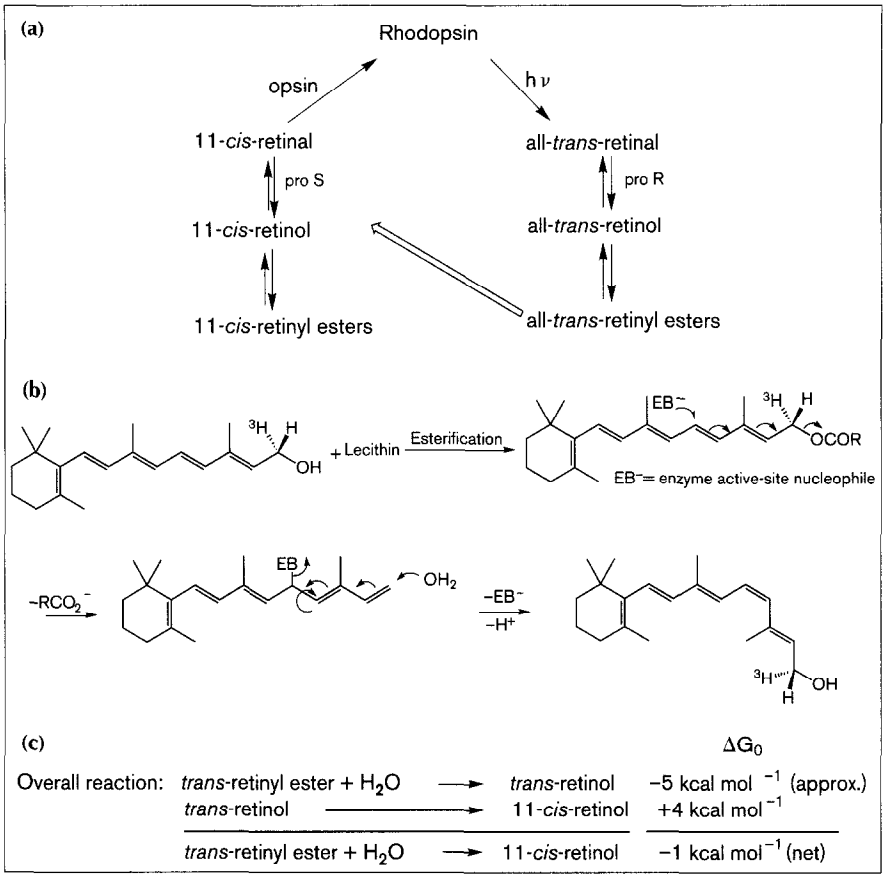
The high energy of 11-*cis*-retinal presents a problem for the completion of the visual cycle: the all-*trans*-retinal formed as a consequence of bleaching must be retransformed into 11-*cis*-retinal, a thermodynamically uphill reaction, to re-start the cycle. How is this achieved?

The all-*trans*-retinal liberated by the bleaching of rhodopsin is rapidly reduced enzymatically in the retina by specific nicotinamide-linked retinol dehydrogenases, producing vitamin A (all-*trans*-retinol). The vitamin A is transported from the rod outer segments to the pigment epithelium (the tissue that sits behind the retina), where it is esterified, largely to long-chain saturated fatty acid esters, such as those in the palmitate and stearate series. For each of the three all-*trans*-retinoids, there is a corresponding 11-*cis*-retinoid. The biochemical reactions required to interconvert these six molecules comprise the visual cycle, shown in simplified form in Figure 6a.

As simple catalysis would provide only 0.1 % of 11-*cis*-retinoids at equilibrium, the biosynthetic pathway for 11-*cis*-retinoids requires an energy-transducing enzymatic step.This step is interesting, as it couples the free energy of hydrolysis of retinyl esters to the thermodynamically uphill isomerization step (Fig. 6b) [14]. Acyl esters are

Figure 6

A current simplified model of the visual cycle. **(a)** Following the conversion of 11-*cis*-retinal to all-*trans*-retinal by light, the enzyme proR yields the alcohol all-*trans*-retinol (vitamin A), which is esterified to the membrane phospholipid lecithin. The isomerization of the retinoid is coupled to the hydrolysis of the ester (although the order of these steps has not been determined) to give 11-*cis*-retinal. **(b)** The reaction mechanism of the esterification to lecithin and the subsequent hydrolysis. **(c)** The unfavorable isomerization reaction is driven by the favorability of the hydrolysis of the ester.



'high energy' compounds; their free energies of hydrolysis are in the ~5 kcal mol^{-1} range. This is more than enough to drive the isomerization process, which requires 4.1 kcal mol^{-1} (Fig. 6c). As predicted by this mechanism, labeling experiments show that the original oxygen of the vitamin A is lost during isomerization. In addition, inversion of the absolute configuration of the prochiral $\underline{CH_2}OH$ accompanies isomerization. If ester hydrolysis occurs simultaneously with isomerization, then the enzyme catalyzing the isomerization reaction is an isomerohydrolase rather than a simple isomerase (Fig. 7), because the substrate and product of the enzymatic reaction are not isomers of one another.

Thus the retinyl esters are not simply storage forms of vitamin A in the eye; instead, they are an important energy source for the visual cycle. Since the retinyl esters are generated by the transesterification of all-*trans*-retinol with phospholipids such as lecithin [13,14], the energy that allows isomerization originates in the membrane phospholipids [15].

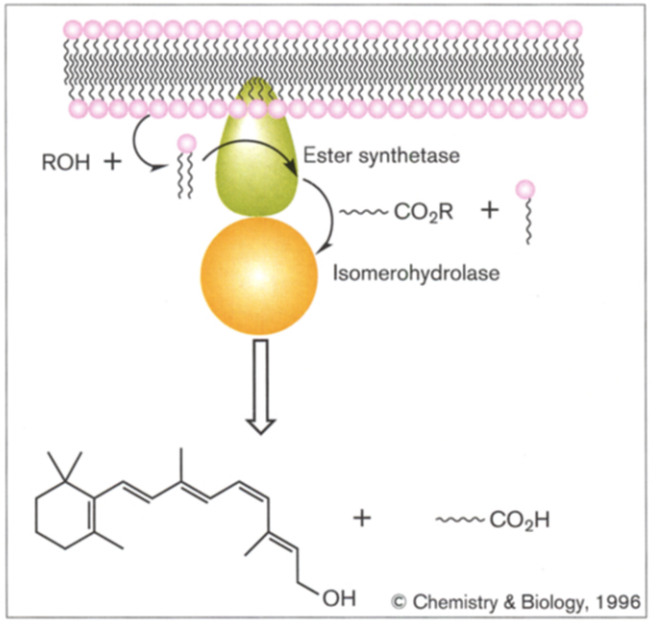
The fact that the regeneration of 11-*cis*-retinal is so complicated again suggests that this molecule has features that are essential for its function in vision. If 13-*cis*-retinal were used as the chromophore, as it is in the non-sensory bacteriorhodopsin (which uses light energy to pump protons in the bacterium *Halobacterium halobium*), then a simple, non-energy-transducing enzyme would suffice to regenerate it.

Why 11-*cis*-retinal?

Which of the characteristics of rhodopsin are important in its visual function, and which of these are due to the special characteristics of 11-*cis*-retinal? The high quantum yield of photoisomerization, the low dark noise, the persistence of the bleached state and the ease of wavelength regulation are clearly important features. The extreme rapidity of the photoisomerization (< 200 ps) does not seem particularly salient or advantageous, however, since the rate-limiting steps in rhodopsin's biochemical interactions occur in the 100 ms time scale.

The high quantum yield (the percentage of captured photons that lead to isomerization) for the photoisomerization of rhodopsin (0.69) is clearly advantageous for the organism, because it allows for vision at low light intensity. Preliminary determinations of the quantum yields for rhodopsins containing different retinal isomers show that rhodopsin containing 11-*cis*-retinal as the chromophore

Figure 7



An isomerohydrolase regenerates 11-*cis*-retinol. All-*trans*-retinol is esterified by a membrane phospholipid, and the hydrolysis of this ester linkage may be catalyzed by the same enzyme that performs the isomerization (an isomerohydrolase).

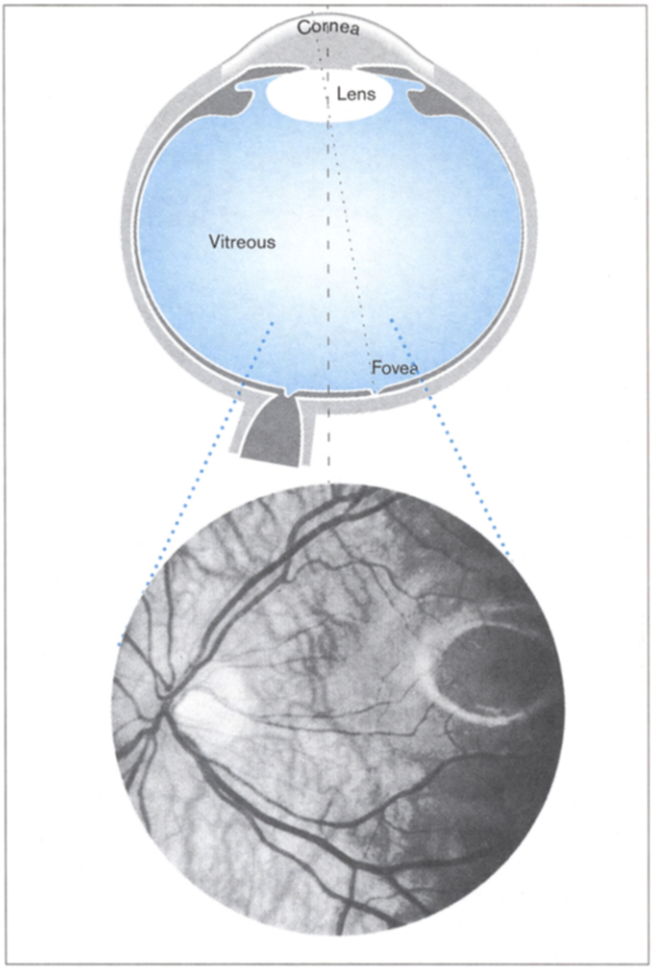
photoisomerizes with a quantum yield three-fold higher than when 9-*cis*-retinal is the chromophore [16]. Thus, this characteristic of rhodopsin may well be explained by the properties of 11-*cis*-retinal.

The low dark noise for rhodopsin isomerization requires that the thermal rate of rhodopsin isomerization be low, and in fact it is low. Superficially it would seem that the strained 11-*cis*-retinal chromophore would be a poor choice for this reason, since it should have a lower activation energy for thermal isomerization than other, less strained, isomers. Although this is certainly true for the retinals in isolation, it may not be for the protein-bound chromophore. The rate of thermal isomerization of the chromophore will clearly be affected by the opsin. The more the protein must move to accommodate the isomerization process, the lower the rate of thermal isomerization will be. The fact that the area swept out during the isomerization of 11-*cis*-retinal is substantially greater than that for the other retinal isomers would then imply that 11-*cis*-retinal should be the least prone to isomerization of the retinal isomers. This line of reasoning is consistent with the finding that large conformational changes in rhodopsin are triggered by isomerization [6].

The thermodynamic instability of 11-*cis*-retinal means that it cannot form spontaneously in significant amounts in the absence of the enzymatic apparatus described above. This aids in the process of photochemical adaptation (visual adaptation after a strong bleach), ensuring that at high light intensities much of the rhodopsin in the photoreceptors remains bleached. The retinals are notoriously sensitive to facile chemical isomerization, and indeed 13-*cis*-retinal (a retinal isomer that has no known physiological function in mammals) is spontaneously generated in the retina during visual processing. A facile back reaction of all-*trans*-retinal to form 11-*cis*-retinal would be problematic for adaptation. As only 0.1 % of 11-*cis*-retinal is found in an equilibrium retinal mixture, however, any possible back reaction is so insignificant that it may be ignored.

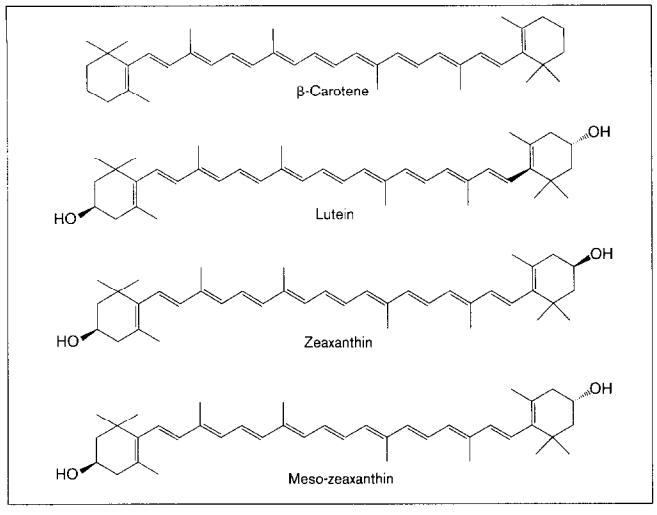
It is often assumed that the relatively high energy of 11-*cis*-retinal (4 kcal mol^{-1} greater than for all-*trans*-retinal) has a second function, in ensuring that the triggering of rhodopsin is a one-way switch. The energy of 11-*cis*-retinal

Figure 8



The structure of the eye. The macula lutea is the area responsible for high-definition, daytime vision, and includes the fovea (top). It is visible as a dark spot (bottom, right) because of the high concentration of the xanthophils zeaxanthin and lutein. The image at the bottom is reprinted, with permission, from [20].

Figure 9



Structures of carotenoids.

is, however, insignificant in this context, given that the energy of the absorbed photons is ~57 kcal mol^{-1}. It is the large energy barrier between metarhodopsin and rhodopsin, not that of the conversion of 11-*cis*-retinal to all-*trans*-retinal, that ensures that the triggering of rhodopsin is an irreversible event.

The final important property of 11-*cis*-retinal as a chromophore relates to spectral tuning, or wavelength control. Since the positive charge on the Schiff base nitrogen is redistributed to the polyene chain in the excited state, it is possible that interactions between charged amino acids in the opsin and the developing charge on the chromophore explain the variation in absorption maxima in the different opsins [11,12]. Thus, suitably placed positively charged amino acids would produce a blue shift, and conversely suitably placed negatively charged amino acids would generate a red shift. It is thus relatively clear why retinals in general might be chosen as chromophores, but it is not clear whether 11-*cis*-retinal is particularly susceptible to wavelength modulation.

The macula pigments

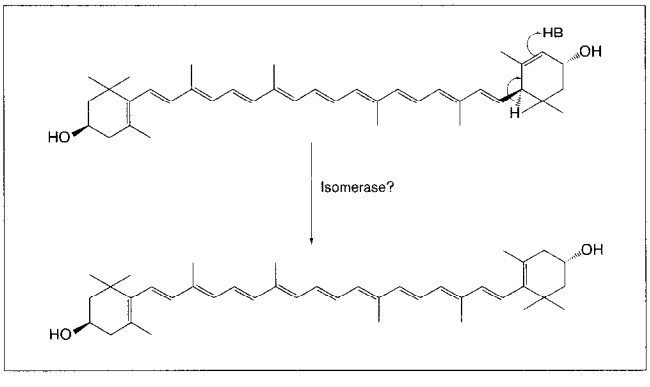
I turn now to the macula lutea, or yellow spot (Fig. 8), the portion of the retina that is most responsible for high-definition, daytime vision [3]. It contains the fovea, where the highest concentration of cones in the retina are found. This part of the retina is exceedingly important in vision. Indeed, age-related macular degeneration (AMD) is an immense public health problem.

As the name implies ('macula' means 'spot, blemish'), the macula is pigmented; it contains the carotenoids zeaxanthin and lutein which give the macula its yellow color (Fig. 9) [3]. These hydroxylated analogs of β-carotene, properly known as xanthophils, are found concentrated in the cone axons of the macula. It has been shown recently that the level of ingestion of foods rich in xanthophils, but not β-carotene itself (which is not a metabolic precursor to the xanthophils in humans), is inversely proportional to the incidence of AMD [2]. Foods rich in xanthophils include spinach and collard greens.

These results have generated much speculation in the literature about the possible importance of the xanthophils in vision. The two main functional proposals considered are that the xanthophils act as optical filters, selectively removing the blue part of the visible spectrum, or that the xanthophils act as anti-oxidants to terminate free radical damage and quench singlet oxygen [3]. Both suggestions have merit, and at this point it is unknown whether either, or both, of these mechanisms operate. Nevertheless, the chemistry of the xanthophils is intrinsically interesting, and may give insight into their function.

Although β-carotene is the major dietary carotenoid, it is excluded from the macula, even though it has virtually the same absorption spectrum as zeaxanthin and is a better free-radical trap and singlet-oxygen quencher than either of the xanthophils [17]. Why were the xanthophils selected instead of β-carotene? To answer this question, we must look to the structural differences between these molecules. Most importantly, the xanthophils are hydroxylated and have polar ends. Moreover, the retinal zeaxanthins have unusual stereochemistry. The normal dietary zeaxanthin is (3*R*,3'*R*)-β,β-carotene-3,3'-diol, and only this stereoisomer is found in human plasma [18]. Yet the zeaxanthins in the macula are roughly a 1:1 mixture of the standard dietary isomer and meso-zeaxanthin, ((3*R*,3'*S*)-β,β-carotene-3,3'-diol) [18]. Thus, whatever the function of the xanthophils in the macula, it seems that the stereochemistry of the molecule is likely to be important. The metabolic pathway for the synthesis of the meso compound in the retina has not yet been identified, but a

Figure 10



A lutein isomerase could produce meso-zeaxanthin.

possible pathway for generating meso-zeaxanthin from lutein is shown in Figure 10.

One crucial structural difference between the xanthophils and β-carotene is that the xanthophils are amphipathic, with a structure that is somewhat reminiscent of the amphotericins. Amphotericins are polyene-containing antibiotics that form pores in membranes. It remains to be determined if there are any functional similarities between the xanthophils and the amphotericins. The inherent hydrophobicity of the xanthophils, coupled with their polar head groups, suggests that they could interact with bilayer membranes. Indeed, given the enormous concentrations of xanthophils in the macula [3], it is hard to imagine where they would be found other than in the membrane bilayer. The question is how these molecules would be distributed in the membrane. The distance between the hydroxyl groups of the xanthophils is ~30 Å, a distance that would not appear sufficient to span the lipid bilayer of a membrane (~50 Å). Biophysical measurements on xanthophil–phospholipid bilayer membranes suggest that the xanthophils adopt defined structures in membranes, and that these structures appear to form an acute angle to the plane of the membrane [19].

If xanthophil molecules are to form discrete structures in membranes, they must be able to interact with each other in defined ways within the membrane. This is consistent with the fact that the unusual epimer meso-zeaxanthin has been observed in the macula. Structural studies on the interactions of the various xanthophil isomers with synthetic bilayer and macula membranes will be required to begin to address the issue of the role of xanthophils in preserving neurons from light and/or oxidative damage. An understanding of these structure–function relationships could lead to the design of novel neuronal protecting agents, which might preserve neurons from destruction not only in the retina, but also possibly in the central and peripheral nervous systems.

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