Pure & Appl. Chem., Vol. 69, No. 10, pp. 2091–2098, 1997.
Printed in Great Britain.

Photo-excitation by a half-carotenoid: Symbiosis between retinal and the visual protein opsin

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Abstract: The visual process is initiated by photoactivation of a receptor protein, e.g. the vertebrate rod visual pigment rhodopsin. Upon absorption of light its chromophore, 11-*cis* retinal, stereoisomerizes to the all-*trans* (AT) state. This conformational change triggers subsequent subtle conformational changes in the protein (opsin), which pass through several distinct intermediate states (photocascade) and within milliseconds culminate in the "active" intermediate (Meta II). The light-dependent trigger reaction basically is a simple 11-*cis* → AT photoisomerization of a retinoid. However, the interaction of 11-*cis* retinal with opsin has bestowed some unique features upon this reaction: 1) it proceeds with unsurpassed speed, and is complete within 200 fs, 2) it is fully stereospecific and 3) it has an unusually high quantum yield (Φ = 0.7). How opsin has adapted to establish such a highly effective symbiosis is still largely unclear. Modern spectroscopic techniques are slowly beginning to unveil some of the secrets of this mechanism. Here, we will address some features of 11-*cis* retinal which seem particularly relevant: its side-chain methyl groups and torsional strain.

INTRODUCTION

The sense of vision has become a very important element in animal physiology. To develop into such a sophisticated tool, several requirements had already to be met in the primary input pathway; the noise level should be very low in the dark state, e.g. in order to allow the vertebrate rod photoreceptor cell to detect single photons; efficient activation and inactivation pathways with high turnover rates are required to allow rapid succession of visual images.

For the primary step in the input pathway, the photosensor, a combination of a photosensitive group (retinal) and a protein (opsin) has evolved into a remarkably efficient and multifunctional molecular system. Retinal belongs to the compound class of retinoids (Fig 1). It can be produced in several body tissues through oxidative cleavage of ß-carotene (ref. 1). Contradictory to its redox analogs retinol (vitamin A) and retinoic acid, which participate in ubiquitous signalling pathways, retinal exclusively functions in the visual process. The 11-*cis* stereoisomer of retinal (refs 2,3), covalently bound in a protonated Schiff base to a lysine residue of opsin (Fig. 2), represents the "dark state" of the light sensor, the photoreceptor protein rhodopsin. This dark state is several orders of magnitude less active than the apoprotein opsin (ref. 5). Hence, the presence of 11-*cis* retinal effectively further suppresses the already low basal activity of opsin and in fact acts as an efficient antagonist. This explains the low noise level.

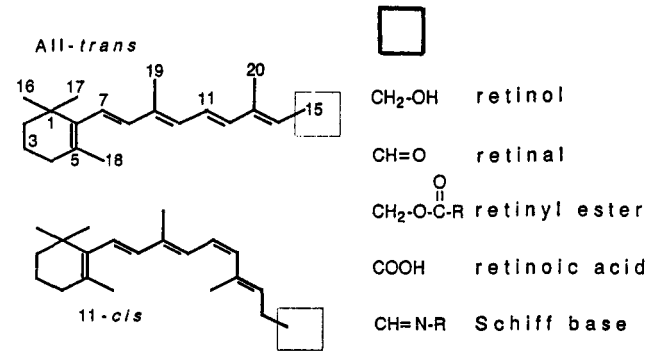

Fig. 1. Major naturally occurring retinoids. Retinal is largely found in the retina in either the all-*trans* (AT) or the 11-*cis* isomeric state.

Upon light absorption, the excited ligand isomerizes to the all-*trans* (AT) isomer and now acts as a full agonist (ref.2). The photo-activated AT-retinal-opsin complex (Rh*) selectively binds and within milliseconds activates a G-protein (transducin); the latter initiates an intracellular transduction pathway which serves to amplify the signal and produces a graded receptor potential, the maximum amplitude of which can already be obtained within ca 50 msec in cone photoreceptor cells, responsible for color vision and image building (ref. 6). Rapid inactivation of Rh* is achieved through phosphorylation by a specific kinase and subsequent binding of an inhibitor, arrestin (ref. 7).

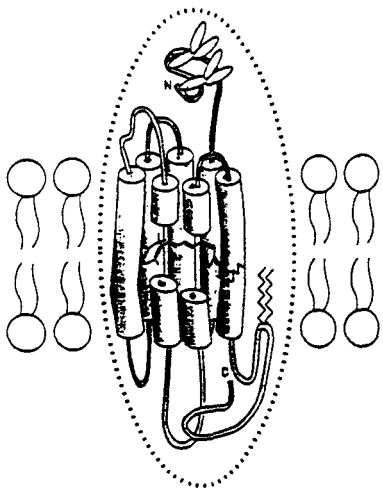

Fig. 2. Schematic model for the structure of bovine rod rhodopsin (adapted from ref. 3). The binding site for 11-*cis* retinal is located within the membrane domain formed by seven trans-membrane helices. A low-resolution 2D projection structure is the best real structure so far available (ref. 4).

Some molecular properties of rhodopsin, in particular the remarkable symbiosis of protein and retinal, will be examined here in more detail.

RETINAL-OPSIN SYMBIOSIS

Retinal-proteins in general

Retinal acts as light sensor (chromophore)/ligand in several photoreceptor systems (Table 1). Examination of their global properties already provides some clues on the molecular properties which render retinal quite an attractive candidate for such a function. All systems bind retinal in a protonated Schiff base. This probably stabilizes the bond against hydrolysis and against thermal isomerization. More importantly, however, the resulting electronic charge distribution can be relatively easily modulated by the protein environment, allowing the absorbance band of its ligand-chromophore to shift away from that of the protonated retinylidene Schiff base in model compounds (ca 440 nm) all over the visible range. This is of particular importance for visual

pigments and has allowed the development of color vision. Also all systems use the absorbed light energy to convert their ligand into another isomeric configuration. The retinochromes, which have been detected so far in insects and cephalopods, probably solely function to produce 11-*cis* retinal as a chromophore for newly synthesized opsin. The other systems, however, utilize this structural change in their ligand to drive the protein conformational changes required for their function. The employment of different isomeric transitions (AT → 13-*cis* in archaerhodopsins; 11-*cis* → AT in visual pigments) probably reflects the fact that for activation of an ion channel smaller and more local changes need to be imposed on the protein conformation than for eventual exposure of binding sites for a G-protein. On the other hand, it might be necessary for the ligand in visual pigments, in order to function effectively as an antagonist in the dark state and as an agonist upon photoisomerization, to adopt isomeric states which require a larger structural transition. The question of how this structural transition in a ligand embedded in the membrane domain is able to drive subtle changes in protein conformation culminating in exposure of G-protein binding sites at the intracellular domain is a very intriguing one, both from a scientific viewpoint and in view of the implications for protein engineering.

TABLE 1: General properties of retinal proteins

	Function	*Absorbance range (nm)*	*Binding mode*	*Trigger reaction*
Archaerhodopsins	Ion translocation	480 - 600	Protonated Schiff base	AT ⇒ 13-*cis*
Chlamyrhodopsin(s)	?	≈ 500	"	AT ⇒ 13-*cis*
Retinochromes	Photo-isomerase	440 - 500	"	AT ⇒ 11-*cis*
Visual pigments	G-protein coupled receptor	350 - 620	"	11-*cis* ⇒ AT

Rhodopsin photocascade

Upon photoexcitation of rhodopsin at physiological temperature, the active intermediate Meta II is generated within milliseconds. However, this is not a single conformational transition but proceeds through a series of at least four discrete, structurally and spectrally distinct, intermediates (photocascade: Rh → Batho → Lumi → Meta I → Meta II) of which only the first transition is light-dependent (Fig. 3). The subsequent transitions represent thermal relaxation in ligand and protein. The presence of such intermediate states of course offers unique access to study the molecular mechanism of this activation process. Unfortunately, detailed structural information on the retinal binding site is not available. Only very low resolution 3D-crystals of rhodopsin have so far been obtained (refs 8,9) and a low resolution 2D-projection structure has been produced (ref.4).

The best structural information so far has been obtained through vibrational spectroscopy, e.g. resonance Raman (which mainly probes the chromophore) and FT-IR spectroscopy (which probes both chromophore and protein) (refs 10,11). In particular FT-IR difference spectroscopy of the various transitions (Rh → Batho, Rh → Lumi etc) is very powerful in this respect, since it monitors structural changes in chromophore and protein, which are accompanied by shifts in vibrational peak frequency, band width or band intensity. Thus, the various transitions present very characteristic difference spectra (Fig. 4). Peak identification is generally achieved through site-specific mutagenesis or, more appropriately, through isotope labelling ($^{1}H \rightarrow {}^{2}H$; $^{12}C \rightarrow {}^{13}C$; $^{14}N \rightarrow$

^{15}N etc.). In this way all major peaks in the 1600 - 800 cm^{-1} region of the Rh → Batho transition have been demonstrated to derive from the chromophore (ref. 10). Specifically, the peaks in the fingerprint region (1300 - 1100 cm^{-1}) present unequivocal evidence that the 11-*cis* → AT isomerization is completed during this transition. Ultrafast (fs) visible spectroscopy shows that the Rh → Batho transition is already finished within 200 fs! (ref. 15). Hence, the symbiosis between retinal and opsin leads to an unprecedented photoisomerization rate. In addition, it provides unusual stereoselectivity and a very high quantum yield ($\Phi \approx 0.7$) (ref. 2). An unusual feature in the Rh → Batho spectrum is the appearance of strong peaks in the 1000 - 800 cm^{-1} region. They have been assigned to single hydrogen-out-of-plane (HOOP) vibrations of the retinal side chain (ref. 16) and can only be explained by assuming that in Batho the retinal is constrained in a highly strained AT-configuration, which only relaxes in the following transition. The subsequent transitions also present a gradual increase in protein activity, supporting the concept that discrete conformational transitions in the protein propagate and culminate at the Meta II stage.

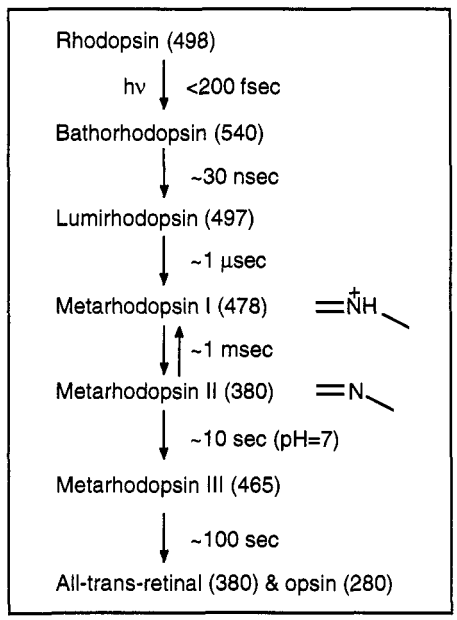

Fig. 3. The rhodopsin photocascade. Time constants are given for room temperature. The cascade can be arrested at a specific intermediate by lowering the temperature to a specific range (e.g. Fig. 4)

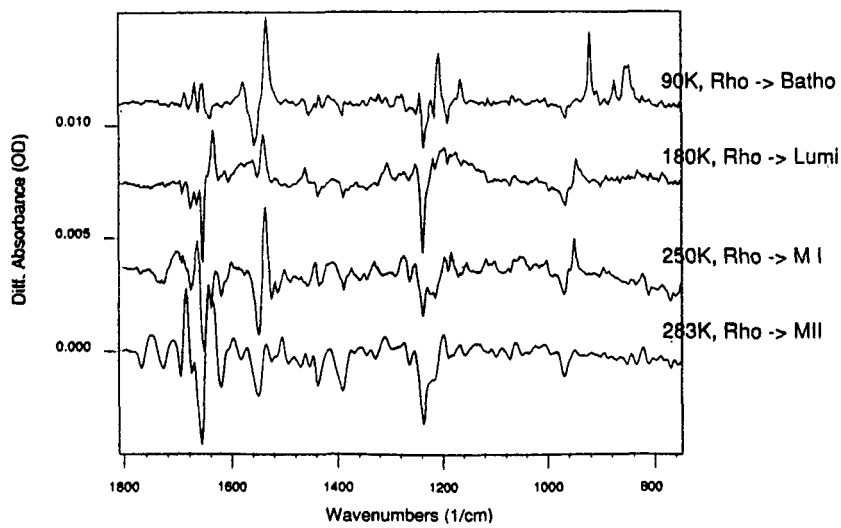

Fig. 4. FT-IR difference spectra of the various transitions in the rhodopsin photocascade (MI = Meta I, MII = Meta II). The spectra were taken at the indicated temperature to arrest the cascade at that specific intermediate and obtained by subtracting the Rho-spectrum from the photoproduct spectrum. Hence, negative peaks represent vibrational bands originally present in rhodopsin, which have changed during the transition. Positive peaks represent bands newly arising in the photointermediate.

Hence, the picture emerges that, at the Batho stage, close contacts occur between the isomerized ligand and opsin which hold the former in a strained conformation and are necessary to initiate the subsequent conformational transitions in the latter. Immediately, the question then arises which molecular elements could be responsible for such close encounters. Due to the lack of structural details this question is not yet easily addressed from the protein side, but from the chromophore side the protruding side-chain methyl groups of course are potential candidates. Indeed, studies on rhodopsin analogs containing methyl-group homologs of 11-*cis* retinal have already been very informative.

Rhodopsins containing (de)methyl-11-*cis*-retinal analogs

Single demethyl analogs of rhodopsin (9-demethyl and 13-demethyl) have been examined before and show profound effects on rhodopsin functionality (refs 17-23). Hence, we have started to evaluate single methyl-group additions and to analyse one of the probably most critical positions: C_{10} (De Lange et al; in preparation). The 10-methyl homolog of 11-*cis* retinal was shown before to produce a visual pigment analog (ref. 23). Structures of the compounds are presented in Fig. 5.

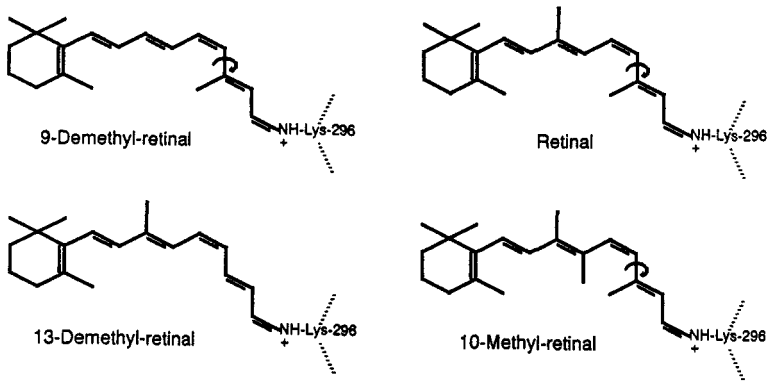

Fig. 5. Schematic structure of single methyl-group homologs of 11-*cis* retinal in the corresponding analog pigment. In the 13-demethyl derivative the torsional strain around the C_{12}-C_{13} single bond is probably relieved.

Some relevant results are compiled in Table 2. The data for the 9- and 13-demethyl compounds were obtained from the literature (refs 17-23). All analogs exhibit substantial perturbation of several rhodopsin properties. The 9-demethyl analog shows a large blue-shift, probably due to the loss of the electron-donating potential of the methyl group, exemplifying that the electronic properties of the chromophore are indeed relatively easily modulated. In addition, this analog has a fully pertubed photocascade, where a clear Batho stage has not yet been identified and the Meta II-like stage is not deprotonated and shows unusual structural features. This might explain the low rate of G-protein activation by this Meta II analog, which is, however, partly counteracted by its low rate of phosphorylation, resulting in significantly slower inactivation. With the 13-demethyl analog also a blue shift in the absorbance band is expected, but this is probably largely compensated by the relief of torsional strain around the C_{12}-C_{13} single bond (see below), which increases conjugation and produces a red shift. Isomerization rate and quantum yield are significantly reduced in this analog. Nevertheless, a distinct but shifted HOOP-pattern in the Batho intermediate is indicative of a strained AT-chromophore, but probably less so than in the native pigment. The photocascade further basically shows normal spectral and structural features and produces a Meta II form with native activity. Most interestingly, however, the dark state also is capable of significant G-protein activation. The addition of a methyl group in the 10-methyl analog produces a red shift, as expected, but whether this is partly counterbalanced by an increase in the C_{12}-C_{13} torsional angle is presently under investigation. This analog effects a very strong perturbation of the photocascade kinetics. Although the HOOP-pattern indicates a strongly strained AT-chromophore at the Batho stage (Fig. 6) and the structural features of the other transitions also are

basically normal, all transition temperatures are substantially shifted upwards. In addition, the kinetics of the Meta I → Meta II transition are profoundly affected. Native Meta I decays within milliseconds at 0°C, but 10-methyl-Meta I is almost stable at 10°C and has a half-time of several minutes at physiological temperature. Analysis of other functional properties of this analog pigment are in progress.

TABLE 2: Single methyl-group homologs of retinal.
Effects on rhodopsin functionality. ? = No data yet available

Chromophore	λ_{max} *(nm)*	*isomerization time (fs)*	*quantum yield*	*strain (batho)*	*photocascade*	*G-protein activation*	
						dark	*light*
11-*cis* retinal	498	200	0.7	++		-	++
9-demethyl-11-*cis* retinal	465	?	?	?	abnormal	-	±
13-demethyl-11-*cis* retinal	495	400	0.3 - 0.5	+	largely normal	+	++
10-methyl-11-*cis* retinal	510	?	0.3	++	strongly slowed down	?	

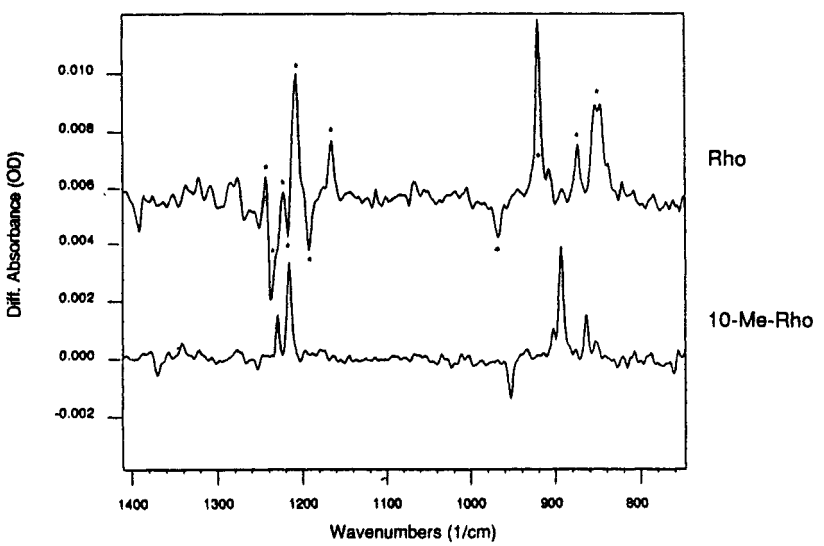

Fig. 6. Rh → Batho difference spectra at 95 K of native rhodopsin (Rho) and 10-methyl-rhodopsin (10-Me-Rho). Asterisks in the Rho spectrum indicate peaks which have been identified as chromophore vibrations (ref. 16).

Clearly, interaction between opsin and its ligand is very finely tuned by evolutionary adaption to get the full benefit from the specific properties of retinal; a beautiful example of molecular symbiosis. The compiled data presented above would allow the very preliminary conclusion that the 9-methyl group is of prime importance for proper triggering of protein conformational transitions during the photocascade, that the 13-methyl group is of prime importance for suppressing structural transitions which could produce thermal activation of the protein ("dark-activity"), and that the C_{10}-position belongs to the critical positions where additional steric hindrance is not

tolerated. However, it is hoped that this picture will be considerably fine-tuned in the coming decade when structural information on a molecular level will become available through FT-IR and solid-state NMR spectroscopy. Initial data on the molecular structure of the chromophore in the binding pocket are presently being collected.

Torsional strain in the chromophore

The present evidence suggests that photo-activation of rhodopsin (Rh*) is preceded by torsional strain in the AT-chromophore at the Batho stage. In addition, torsional strain around single bonds like C_{12}-C_{13} might contribute to the photoisomerization kinetics. Hence, detailed knowledge of the molecular structure of retinal in rhodopsin, as well as in its photocascade intermediates, is of critical importance. Novel developments in solid-state NMR-spectroscopy like rotational resonance (RR) and local double quantum coherence can finally address this issue. Recently we have demonstrated that, in double-^{13}C labeled retinal, distances between two ^{13}C-nuclei up to 4.4 Å can be determined with high accuracy (± 0.06 Å) by RR NMR (Verdegem et al, submitted). From a similar approach on rhodopsins containing double-labeled retinal we already have derived a torsional angle of 42 ± 6° around the C_{12}-C_{13} single bond (Verdegem et al, in preparation), which is somewhat larger than that calculated for model compounds (35-40°; refs 23,24). This might be the first indication in the rhodopsin ligand of additional torsional strain, which, even when the additional strain is limited, could nevertheless contribute to the isomerization rate. Analysis of the 10-methyl analog may shed more light on this fascinating system.

Full benefit of the modern NMR and FT-IR techniques can be taken as soon as protein labeling of these complex membrane proteins can also be accomplished. This approach is in progress in our laboratory and the first requirement, large-scale production of recombinant rhodopsin, has basically been achieved (Klaassen et al, in preparation).

ACKNOWLEDGEMNETS

We thank Dr. Corné Klaassen for his expert help with preparation and printing of several figures.

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