CHEMBIOCHEM

Active States of Rhodopsin

Oliver P. Ernst* and Franz J. Bartl*[a]

KEYWORDS:

G-protein-coupled receptors · IR spectroscopy · photochemistry · protonation · signal transduction

1. Introduction

Rhodopsin, the visual pigment of the vertebrate rod photoreceptor cell, is a G-protein-coupled receptor (GPCR) with seven transmembrane α-helical segments. It defines the rhodopsin-like (class A) family within the large GPCR superfamily[1] and is the only GPCR for which a high-resolution crystal structure has been determined.[2] It is composed of the 40-kDa apoprotein opsin (348 amino acids) and its chromophore, 11-*cis*-retinal, a derivative of vitamin A that acts as an inverse agonist in the rhodopsin ground state. The retinal is covalently bound to the terminal amino group of Lys296 in transmembrane helix VII (H-VII) as a protonated Schiff base (Figure 1) and its spectral absorption characteristics are tuned by the protein moiety to the wavelengths that correspond to green light. Photon absorption by rhodopsin isomerizes the 11-*cis* chromophore to all-*trans*, which triggers a series of conformational changes in opsin and leads to an active state of rhodopsin. This state is competent for binding the heterotrimeric G-protein of the rod cell, transducin (Gt) and for catalysis of the uptake of guanosine triphosphate by the α subunit of Gt, and thereby initiates the enzymatic cascade that leads to vision.[3, 4]

Rhodopsin attracts attention as a prototypical GPCR and as a photoreceptor pigment with its light-induced formation of photointermediates that can be distinguished spectroscopically.[5] The peculiarity of rhodopsin, the photochemical generation of an agonist inside the ligand binding pocket, allows information to be obtained on the activation process of this GPCR. In this work we will review the differences between the late rhodopsin photoproducts and signaling states as investigated by UV/Vis and IR spectroscopy. For additional information on structure–function relationships in rhodopsin and GPCRs in general, the reader is referred to recently published reviews.[6–8]

2. Light-Dependent Activation of Rhodopsin by Photoisomerization

2.1 Ground state and early photoproducts

A striking feature of the ground state of rhodopsin is its inactivity towards nucleotide exchange catalysis in Gt. This was deduced from the very low level of noise of photoreceptor cells, which is mandatory for their function as single quantum detectors.[9] The rhodopsin crystal structure provides an explanation for this characteristic by revealing structural constraints in the ground state.[2, 10] Among these structural features are several hydrogen-bonded networks that constrain the transmembrane helices and the interaction between the inverse agonist 11-*cis*-retinal and its environment. The chromophore is fixed by tight hydrophobic interactions at the β-ionone moiety and polar interactions in the region of the Schiff base, in particular by a stabilizing salt bridge between the protonated Schiff base and the counterion Glu113.[4] The chromophore/protein interaction and protonation of the Schiff base account for the broad visible absorption maximum of rhodopsin ($\lambda_{max} = 500$ nm, Figure 2) compared to the free chromophore ($\lambda_{max} = 380$ nm).

After photon capture and concomitant *cis*/*trans*-isomerization of the chromophore, rhodopsin relaxes through a series of photoproducts that lead to the active conformation, meta II, and eventual decay to the apoprotein opsin and all-*trans*-retinal. The classical photointermediates of rhodopsin, which can be distinguished by UV/Vis and IR (especially FTIR) spectroscopy, are depicted in Figure 3. These spectroscopic techniques are reliable tools for the characterization of discrete intermediates and allow their formation to be monitored.[11, 12] Measurement of molecular vibrations of the chromophore, amino acid side chains, and peptide backbone by FTIR difference spectroscopy allows identification of molecular changes that arise from light-induced retinal isomerization in the chromophore-binding domain. To get an insight into the mechanism of rhodopsin activation, pigments with site-specific mutations and/or modified chromophores were investigated with both techniques and key residues involved in rhodopsin activation were identified.[3, 7, 13]

Highly efficient excitation of rhodopsin by photon absorption (at around 500 nm, quantum yield $\phi = 0.67$) isomerizes the 11-*cis*-ene to the *trans* form, which leads to the primary photoproduct photorhodopsin within 200 femtoseconds.[14] The first high-energy intermediate of the relaxation process that can be stabilized at low temperature ($-140\,^{\circ}C$) is bathorhodopsin ($\lambda_{max} = 543$ nm). This intermediate stores two thirds of the photonic energy (≈ 30 kcal mol^{-1}),[15] and Raman and FTIR difference spectroscopy revealed that the chromopore is in a twisted 11-*trans*,15-anti conformation.[16, 17] The reason for formation of

[a] *Dr. O. P. Ernst, Dr. F. J. Bartl*
Institut für Medizinische Physik und Biophysik
Humboldt-Universität zu Berlin
Medizinische Fakultät Charité
10098 Berlin (Germany)
Fax: (+49) 30-450-524-952
E-mail: oliver.ernst@charite.de, franz.bartl@charite.de

  1439-4227/02/03/10 $ 20.00+.50/0 ChemBioChem **2002**, 3, 968 – 974

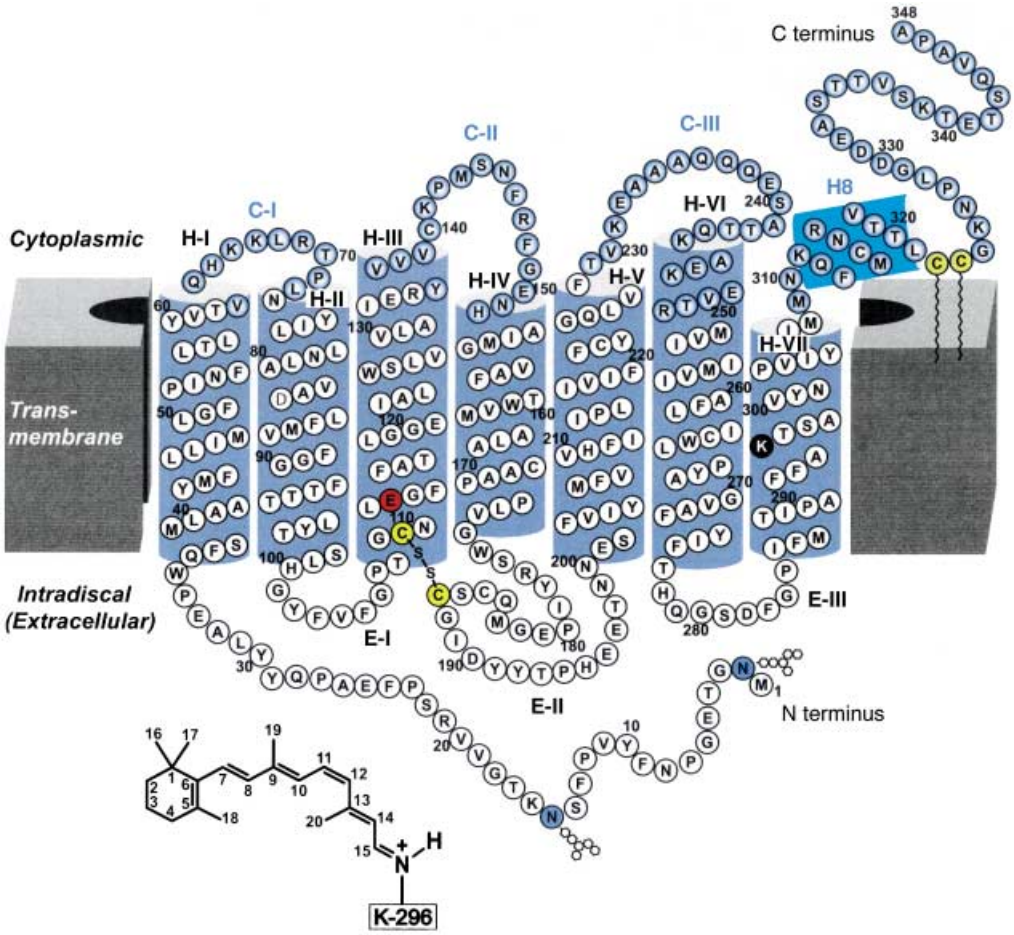

Figure 1. *Secondary structure model based on the crystal structure of bovine rhodopsin.[2] Transmembrane α-helical segments are shown as grey-blue cylinders and are designated H-I to H-VII. The cytoplasmic amphipathic helix H8 (bright blue cylinder) is terminated by palmitoylated Cys322 and Cys323 (filled yellow circles). Lys296 (filled black circle) in H-VII forms a protonated Schiff base with the chromophore, 11-*cis*-retinal. The counterion of the protonated Schiff base is Glu113 (filled red circle). The cytoplasmic domain (top; circles with a blue periphery) comprises peptide loops C-I to C-III, which connect successive helices, and the C-terminal tail. The N-terminal tail and intradiscal domain are toward the bottom of the figure and represent the extracellular side of this GPCR. An essential disulfide bridge between Cys110 and Cys187 stabilizes the inactive receptor conformation. Asn2 and Asn15 at the N-terminus carry carbohydrate chains. Amino acid residues are depicted with the single-letter code. Intradiscal peptide loops, which connect successive helices, are designated E-I to E-III. Bottom: Structure of the chromophore 11-*cis*-retinal, which is bound to the side chain of Lys296 through a protonated Schiff base in rhodopsin.*

this distorted all-*trans* conformation might be a steric restriction caused by approach of the polyene chain of the retinal and the side chain of Ser186 after isomerization around the C11–C12 double bond.[6] This twist seems to occur in the middle of the chromophore since neither the position of the β-ionone ring nor the environment of the Schiff base changes.[11] The chromophore movement causes slight alterations in the backbone structure, as indicated by infrared difference bands in the amide-I region.[17, 18] FTIR difference bands evoked by the protonated carboxylic acid group of the amino acid side chains of membrane-embedded Asp83 in H-II and Glu122 in H-III[19] have only low intensities, which suggests that the environment of these residues changes very little.[20]

Thermal relaxation leads through a blue-shifted intermediate (Figure 3),[5, 21] which does not accumulate at low temperatures and can only be obtained in time-resolved measurements, to lumirhodopsin. In lumirhodopsin, which can be stabilized at $-40\,^{\circ}C$, most of the twist in the polyene chain has relaxed, as seen by the low intensities of the hydrogen-out-of-plane (HOOP) vibrations of the chromophore.[20] The C=N group has moved from an environment in which it was strongly hydrogen-bonded to a region with weak hydrogen bonds. Glu122, which is in the middle of H-III close to the β-ionone moiety of the chromophore, changes its hydrogen bond environment when lumirhodopsin is formed.[20] This change is in agreement with recent photoaffinity labeling studies that used a retinal analogue with two photoactive moieties. The studies concluded that during the conversion from bathorhodopsin into lumirhodopsin, the β-ionone moiety of the chromophore flips over from the nearby Trp265 in H-VI into the vicinity of Ala169 in H-IV.[22] Rhodopsins regenerated with β-ionone-ring-modified retinal analogues showed lower activity towards the G-protein.[23] It may be concluded that the movement of the β-ionone ring triggers the formation of the protonation- and G-protein-dependent meta I and meta II states. These products form on the time scale of micro- and milliseconds.

This conclusion is supported by studies with ring-constrained retinal analogues. These 11-*cis*-retinal analogues have a bridge

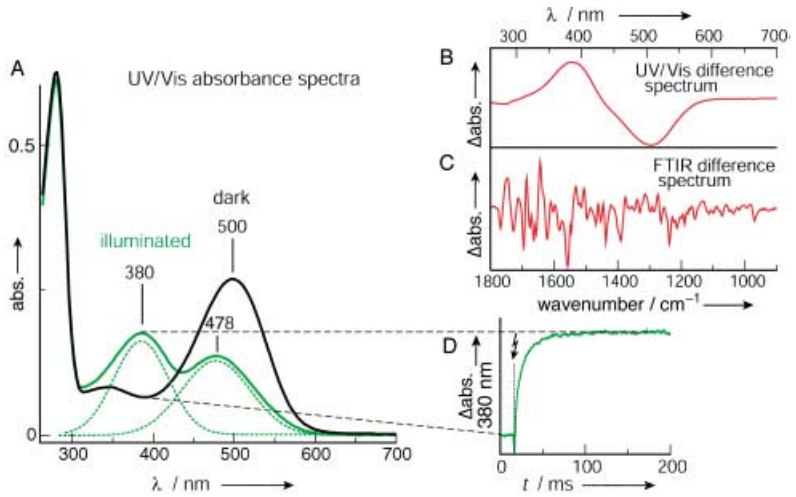

Figure 2. *UV/Vis and IR absorption spectra of rhodopsin. A: The characteristic absorption spectrum of the rhodopsin ground state ($\lambda_{max} = 500$ nm) originates from the interaction between the chromophoric ligand 11-*cis*-retinal ($\lambda_{max} = 380$ nm) and its binding site in the apoprotein opsin. The red-shifted λ_{max} value of rhodopsin is a result of the protonated form of the Schiff base and delocalization of the positive charge throughout the polyene chain, which is affected by the distance of the counterion Glu113 from the Schiff base and the local electrostatic field within the binding site. The absorption at 280 nm represents the apoprotein component. Within milliseconds after illumination at room temperature, a pH-dependent equilibrium between metarhodopsin I (meta I, $\lambda_{max} = 478$ nm) and the G-protein-interacting active state, metarhodopsin II (meta II, $\lambda_{max} = 380$ nm), is reached. Gaussian curves represent the two components. B: The photobleaching UV/Vis difference spectrum (meta-II − rhodopsin) is obtained by simply subtracting the dark spectrum from a (pure meta II) light spectrum and yields information about the protonation state ofthe Schiff base. C: Analogously, an FTIR difference spectrum (meta-II − rhodopsin) is obtained by subtraction of the dark spectrum from the light spectrum. By FTIR vibrational spectroscopy, which measures absorption in the infrared range, information about changes in carboxy groups, the protein backbone, and the chromophore of rhodopsin can be obtained (see Figure 5).[13] D: Time-resolved formation of the meta II state can be followed by flash photolysis.[61] UV/Vis and FTIR spectra were taken at room temperature on identically prepared samples of hypotonically extracted rod outer segment disk membranes from retinae. The pelleted membranes were transferred without drying between two BaF_2 windows (d = 5 μm, about 2.2 mM rhodopsin).[72] UV/Vis spectra were corrected for scattering.*

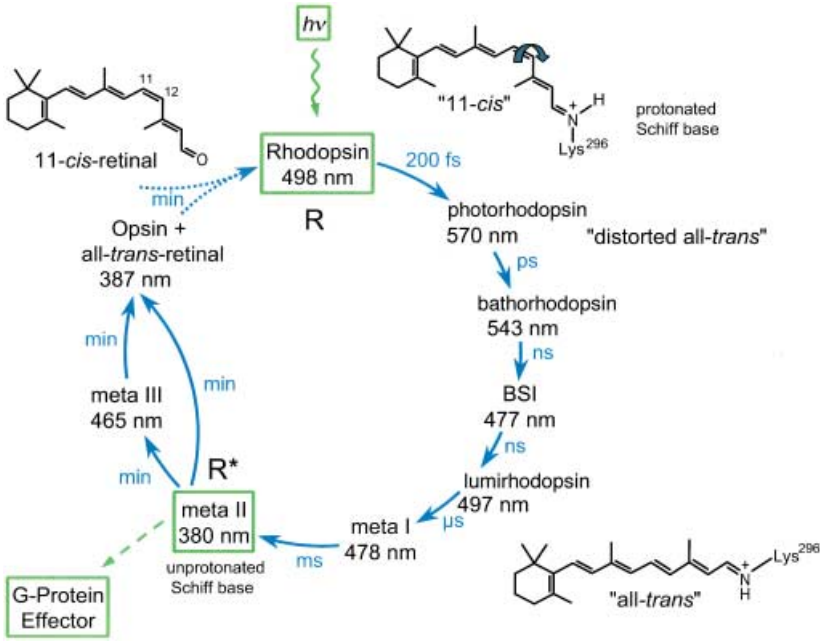

Figure 3. *Photocyle of rhodopsin with spectroscopically detected intermediates. Photoisomerization of the 11-*cis *double bond occurs on a femtosecond time scale and yields the photoproduct photorhodopsin with a highly distorted 11-*trans *bond. The photolyzed pigment thermally relaxes through several intermediates which can be distinguished because of their distinct λ_{max} values by low-temperature spectroscopic studies[73] or flash photolysis at room temperature.[5] Progressive release of the strain in the chromophore leads through batho- and lumirhodopsin to meta I, as seen by the different absorption maxima that arise from changes in chromophore/protein interaction. A blue-shifted intermediate (BSI) cannot be trapped at low temperatures and appears transiently. Up to meta I, protonation of the Schiff base persists, probably due to the low pK_a of the stabilizing counterion Glu113. Larger conformational changes of the protein moiety occur during the transition to the active state meta II,[74] which is in equilibrium with its predecessor meta I. Meta II represents the agonist-bound active receptor state capable of interaction with the appropriate G-protein transducin. Transition to meta II is facilitated by retinal in its all-*trans *conformation, which allows efficient proton transfer to the counterion. Deprotonation of the Schiff base results in a large blue-shifted value for λ_{max} (380 nm). Meta II decays, either directly or through a species termed meta III with a reprotonated retinal Schiff base ($\lambda_{max} = 465$ nm), into the apoprotein opsin and all-*trans*-retinal as a result of irreversible hydrolysis of the Schiff base linkage. Unlike in invertebrates, rhodopsin cannot be regenerated in situ by reisomerization of retinal with a second photon. All-*trans*-retinal is reduced to retinol by retinol dehydrogenase and transported out of the photoreceptor cell to adjacent retinal pigment epithelial cells, where 11-*cis*-retinal is regenerated (for details, see ref. [75]).*

between C10 and C13 that consists of one to four carbon atoms and prevents isomerization around the C11–C12 bond. Rhodopsins reconstituted with these analogues are stabilized in their inactive (or minimally active) conformation even in the light.[24–28] Photoisomerization of these retinals can still occur even if they are reconstituted with opsin, albeit not around the C11=C12 double bond. Illumination of rhodopsin regenerated with an analogue that contains a two-carbon bridge between C10 and C13 (six-membered ring) allows extraction of several isomers (9-*cis* and/or 13-*cis* forms of the ring-constrained 11-*cis*-retinal analogue).[28] However, Gt activation assays and FTIR spectroscopy investigations indicate that this photoisomerization does not result in significant activation of transducin[26–28] or in chromophore-induced activating structural changes in the opsin moiety.[28] Figure 4 shows an overlay of retinal in its *cis* and all-*trans* conformations and a locked retinal analogue in its 9,11,13-tri-*cis* and 11,13-di-*cis* conformations, modeled in the rhodopsin ground state structure. Correct repositioning of the β-ionone ring (which does not occur with the locked retinal analogue) in the course of *cis*/*trans* isomerization was suggested to be a decisive step towards receptor activation.[28]

2.2 The metarhodopsin states

The meta I intermediate is in a pH- and temperature-dependent equilibrium with its successor meta II.[3] Meta I can be stabilized by either high pH values or low temperature. According to IR spectroscopy results, meta I is the first intermediate in which large changes occur in the secondary structure of the protein. This conclusion was reached from the intensity gain of the FTIR spectroscopy amide-I bands, which are sensitive to structural changes.[20] It can also be deduced that in meta I and lumirhodopsin the distortion of the chromophore is similar, and that the Schiff base proton is weakly hydrogen-bonded in meta I (which accounts for a lower pK_a compared to the ground state).[20]

Transition to the signaling state meta II, which binds and activates the G-protein,[3] is accompanied by a large shift of the absorption maximum from 478 nm in meta I to 380 nm in meta II, as a result of deprotonation of the Schiff base (Figure 3). In addition, protonation of the counterion Glu113 occurs, which suggests a proton transfer in the hydrophobic core of the protein.[29] FTIR difference spectra indicate that the largest light-induced alterations of the apoprotein structure take place upon meta II formation.[18] An extensive approach that used site-directed spin-labeling of the cytoplasmic surface of rhodopsin and EPR spectroscopy led conformational changes of the protein moiety to be attributed to an 8-Å displacement of H-VI outward toward residue Val227 on H-V and an outward displacement of H-II relative to H8 of 3 Å.[30, 31] Conformational changes in the protein interior are sensed by Asp83 and Glu122, which are more or less strongly hydrogen-bonded, respectively, in meta II compared to the ground state.[19] Proton transfer reactions are, in general, indicators of structural changes since they occur only if factors like relative orientation and distance of the donor and acceptor groups that form the hydrogen bonds are appropriate.[32] It was found that the 9-methyl group of retinal is necessary for the structural changes that lead to active rhodopsin.[33, 34] It was suggested that all-*trans*-retinal provides the scaffold for the correct adjustment of donor/acceptor groups, which allow structurally sensitive proton transfers.[33] The conformational change affects the environment of the protonated Schiff base such that its pK_a value is lowered from 16 to about 2.5 in meta II.[35] The proton transfer from the protonated Schiff base destroys the salt bridge; the salt bridge is one of the crucial stabilizing elements of the ground state and, in consequence, its destruction may trigger the helix movements that lead to the active, G-protein-interacting conformation of the cytoplasmic surface of rhodopsin. It was found that Schiff base deprotonation is followed by a second protonation step—proton uptake by the cytoplasmic surface. The fact that the two events can be separated by a 20-ms time interval in detergent solution indicated the existence of two isospectral meta II forms, meta II_a and its successor, meta II_b.[36] The likely proton acceptor is Glu134 at the cytoplasmic end of H-III (Figure 1) since proton uptake is controlled by this residue.[37] Spectroscopic studies showed that this residue is protonated in the complex between rhodopsin and transducin.[38] Finally the light-induced events result in decay of meta II into opsin and all-*trans*-retinal by two parallel pathways, either directly or through meta III, in which the Schiff base is protonated again.

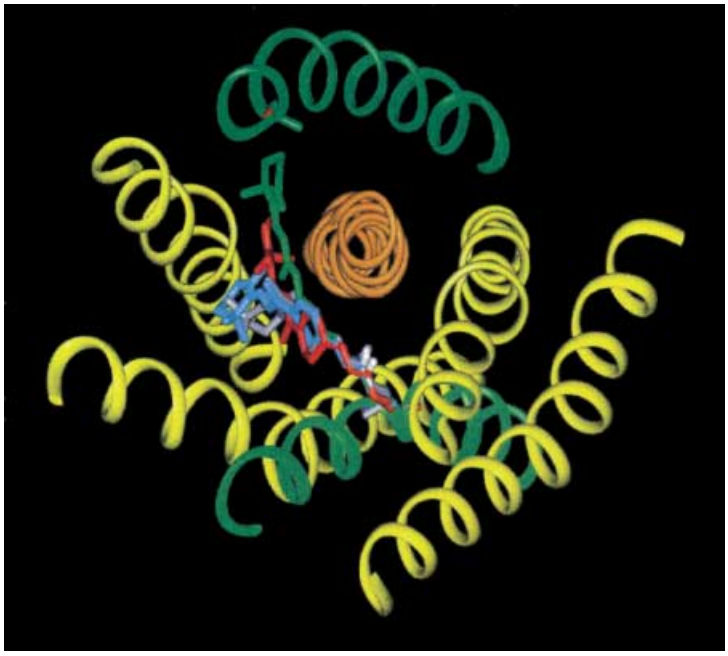

Figure 4. *Structure of retinal isomers and 11-*cis*-locked retinal analogue isomers in the binding site of opsin. Rhodopsin ground state with 11-*cis*-retinal (blue, shown as a stick model) viewed from the intradiscal side. All-*trans*-retinal (green) and two ring-constrained 11-*cis*-retinal analogues (9,11,13-tri-*cis*-locked retinal analogue (gray) and 11,13-di-*cis*-locked retinal analogue (red)), which form upon illumination of the 11-*cis* isomers, are also modeled into the retinal binding site and superimposed. After* cis/trans *isomerization around the C11–C12 double bond and repositioning of the* β*-ionone ring, C3 of retinal is close to Ala169 (marked in red) in H-IV (green, top). Reproduced from ref. [29] with permission. Copyright (2001) The American Society for Biochemistry and Molecular Biology.*

How are the metarhodopsin states related to activation schemes of GPCRs? An analogy can be made between the meta I and meta II states of rhodopsin and the low and high affinity binding conformations of ligand-activated GPCRs, as shown in Scheme 1. The corresponding high affinity conformation of rhodopsin, meta II_b, is either stabilized by direct proton uptake from the aqueous phase, most likely by protonation of the Glu134 side chain in the highly conserved Asp(Glu)-Arg-Tyr motif in H-III, or by binding of the G-protein, which may induce protonation of this residue. Analogous proton translocation reactions have been proposed for other GPCRs.[39–41] This stabilizing effect of Glu134 protonation on the active state becomes obvious when looking at the retinal analogue 9-demethyl retinal, which lacks the 9-methyl group. This retinal shows partial agonism.[33, 34] However, when reconstituted into the E134Q mutant, full activity was observed, as compared to wildtype rhodopsin with normal retinal.[33] This mutation (or a low pH value) shifts the coupled equilibrium towards the active meta II_b conformation and rescues the transition from meta I to meta II in the 9-demethyl retinal pigment, which is impaired by a less efficient proton transfer from the retinal Schiff base.

Rhodopsin	light-induced pathway	→	meta I + G	⇌	meta II_a + G	⇌ meta II_b•G
GPCRs	L + R + G	⇌	LR' + G	⇌	LR* + G	⇌ LR^*•G

Scheme 1. *The analogy between the meta I and meta II states of rhodopsin and the low- and high-affinity binding conformations (R′ and R*, respectively) of ligand (L) activated GPCRs. G = G protein. R = GPCR.*

As mentioned above, the formation of the active state is accompanied by several proton transfer reactions. Whether these protonation changes occur by direct proton transfer from the donor to the acceptor group or through bridging water molecules is not clear at the moment, but there is spectroscopic evidence that water plays a decisive role in the activation process of rhodopsin. It was observed by FTIR difference spectroscopy that air-dried rhodopsin forms lumirhodopsin, which is structurally comparable to the lumirhodopsin obtained from hydrated samples.[42] However, the formation of the later intermediates, meta I and meta II, is affected.[42, 43] It was reported that several water molecules undergo changes in hydrogen bonding upon formation of meta I or meta II,[44] which may explain why the structural changes that lead to metarhodopsin I are partially inhibited under dry conditions.[42]

Spectroscopic studies proposed the existence of water molecules close to the residues Glu113[45] and Asp83.[46] Crystallographic work on the rhodopsin ground state has so far identified seven water molecules in a structural model refined to 2.6-Å resolution.[2, 10, 47] Water molecules are involved in stabilization of the ground state by mediation of interactions between H-I and H-II, and H-VI and H-VII, respectively. Three water molecules are part of a hydrogen-bonded network that constrains H-II (through Asp83), H-III, H-VI, and H-VII. Two water molecules were observed in the retinal binding pocket and are involved in spectral tuning. A water molecule which is close to Glu113 does not reside between Glu113 and the protonated Schiff base but is thought to stabilize this salt bridge by lowering the pK_a value of Glu113.[47]

3. Light-Independent Signaling

The activity of ligand-free opsin towards transducin is very low and is only about $1/10^6$ of the activity of meta II.[48] It was concluded from FTIR spectroscopy investigations that opsin exists in a pH-dependent equilibrium between an inactive conformation at neutral pH values and a conformation very similar to meta II at pH 4.0. The latter conformation forms as a result of protonation of Glu113 and concomitant breaking of the putative stabilizing salt bridge between Lys296 and Glu113.[49] A nonvanishing population of this active conformation of opsin may be the molecular reason for the small but existent activity of opsin at neutral pH values.[49] Enhanced constitutive activity, the ability to activate transducin in the absence of any chromophore, is displayed by some mutant opsins. This enhancement can be caused by a loss of the stabilizing salt bridge as a result of mutations of Lys296 or Glu113.[50] Mutations in or close to the cytoplasmic surface can also result in constitutive activity as seen, for example, with neutralizing mutations of Glu134 at the cytoplasmic end of H-III[51] or a mutation of Met257 in H-VI.[52] It is noteworthy that binding of 11-*cis*-retinal can reduce the constitutive activity of these mutants dramatically because of the activity-inhibiting potential of the inverse agonist.

Light-independent highly active forms of native opsin can be generated by the mere addition of all-*trans*-retinal to the apoprotein opsin.[53–55] This opsin·all-*trans*-retinal complex ($\lambda_{max} = 380$ nm) is orders of magnitude more active than opsin and gains approximately 50% of the activity of meta II at pH 7.[55] The active opsin·all-*trans*-retinal complex is also formed with permethylated opsin, which lacks free lysine side chains. This and other lines of evidence suggest a noncovalent binding of all-*trans*-retinal to opsin rather than the formation of a covalent meta-II-like deprotonated Schiff base between opsin and all-*trans*-retinal.[54, 55] It was found that palmitoylation of Cys322–Cys323 is important for interaction of Gt with this light-independent signaling state, which suggests that these cysteine residues and the adjacent H8 are part of a second binding site for all-*trans*-retinal.[55]

4. Photoregeneration of Rhodopsin

Attempts to reverse the normal activating pathway by photolysis of meta II with blue light ($\lambda < 420$ nm) result in a shift of the absorption maximum, which indicates reprotonation of the retinal Schiff base and proton release. It was shown by UV/Vis spectroscopy that photolysis of meta II leads to a mixture of two photoproducts with absorption maxima of 500 nm and 470 nm, respectively (Figure 5 A). The formation proceeds by two parallel

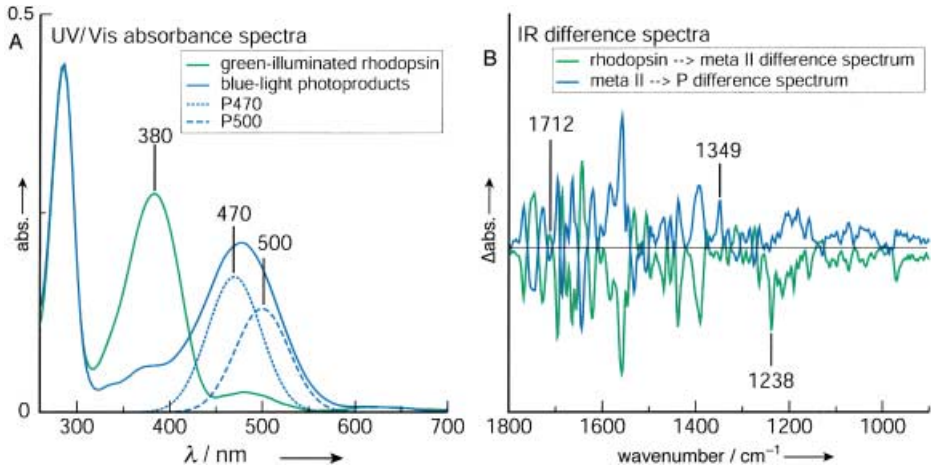

Figure 5. *Absorption change after photolysis of the active state meta II with blue light. A: Photolysis of the active intermediate meta II ($\lambda_{max} = 380$ nm, spectrum plotted in green; compare with Figure 2) with 400 nm light leads to a mixture of two photoproducts (P-products, spectrum plotted in blue) with absorption maxima at 500 nm (P_{500}) and 470 nm (P_{470}), respectively,[57] and different kinetics of formation.[56] The two products, P_{500} and P_{470}, are individually represented by Gaussian curves. B: FTIR difference spectra of the light-induced transitions. The green line represents the difference spectrum of meta II minus rhodopsin. Negative bands are from vibrations of rhodopsin and positive bands are from vibrations in meta II. Blue shows the difference spectrum of the P-products minus meta II. Negative bands correspond to meta II and positive bands to the P-products. A comparison of the two spectra shows clearly that spectral features arising from the protein moiety (protein bands) are reversed in the spectrum that involves the products, which suggests a conformational change back to the ground state. These spectral features reflect structural alterations in the protein that comprise changes in hydrogen bonding and protonation of carboxy groups (Asp83, Glu122, and Glu113, at 1768 cm^{-1}, 1748 cm^{-1}, and 1712 cm^{-1}), and in the peptide backbone (amide I and amide II bands at 1700 – 1620 cm^{-1} and 1570 – 1500 cm^{-1}, respectively). No reversal, however, is seen for a second class of bands (retinal bands), which arise from changes in retinal geometry and retinal – protein interaction. This observation is reflected in C–C stretching vibrations (fingerprint region; 1238 cm^{-1} band and its satellites), C=C stretching vibrations (1550 – 1570 cm^{-1}), and HOOP vibrations (960/970 cm^{-1}). A new band at 1349 cm^{-1} is specific for meta II photolysis. This indicates that the geometry of the retinal and its interactions with the chromophore are different in the P-products from in the ground state.*

pathways with fast and slow kinetics, which lead to the products P_{470} (1 ms) and P_{500} (50 ms), respectively.[56, 57] These P-products were identified by their absorption maxima as the 11-*cis* or 9-*cis* ground state.[56–60] The P_{500} photoproduct cannot interact with transducin, which allows flash photolysis to be used to investigate the interaction of Gt with the active state.[56, 59, 61]

A fundamental change in our understanding of photolysis of the active state arose from a recent study, which showed that the P_{500} photoproduct does not represent the ground state, but rather a product with new properties.[62] Figure 5B shows the FTIR difference spectra of the rhodopsin →meta II conversion (green line; photolysis of rhodopsin with green light) and the meta II →P-products conversion (blue line; photolysis of meta II with blue light). The two spectra represent a perfect mirror image of each other within the spectral ranges observed, which reflect changes in the protonation states of carboxylic acids, hydrogen bonding, and secondary structure. This result, however, does not apply to the spectral region indicative of the retinal geometry. The exact conformation of retinal in P_{500} straight after photolysis of meta II and its exact interactions with the protein environment are still open questions. Among other possibilities, it was suggested that the conformation could even be all-*trans* because mainly all-*trans*-retinal was extracted from photolyzed meta II, as identified by HPLC analysis.[62] Taken together, it is evident that P_{500} does not represent the ground state, but has many of the characteristics of that state. The IR spectra of P_{500} and meta III, which arises from meta II by thermal decay, are similar. Furthermore, FTIR spectroscopy suggests that both species, P_{500} and meta III, undergo similar conformational changes upon photolysis with green light, to give a meta-II-like species.[62] It remains to be investigated how these findings are connected with blue-light-induced degeneration of the retina,[63] bleaching adaptation, and the potential physiological role of meta III as a storage conformation.[3, 64]

5. Interaction of Photoactivated Rhodopsin with Transducin

UV/Vis spectroscopy experiments that make use of the intrinsic reporter group of rhodopsin for the transition to the active conformation, the retinal Schiff base, have shown that bound G-protein stabilizes meta II at the expense of inactive meta I, to produce "extra meta II".[65] In this regard, the Gt holoprotein can be replaced by synthetic peptides from the Gtα and Gtγ C-terminal sequences, which suggests that the respective structures of transducin are interaction sites for rhodopsin.[66–68] Interaction of activated rhodopsins with Gt and Gtα- and Gtγ-derived peptides was also detected by flash photolysis of the respective complexes[59] or by FTIR spectroscopy in transmission or attenuated total reflection modes.[69–72] Both Gtα- and Gtγ-derived peptides stabilize (at basic pH values) the meta II form, which is formed spontaneously under acidic pH conditions,[72] and protonation of a carboxylic acid upon binding of Gt was assigned to Glu134.[38] Although the conformational changes induced upon binding of the Gtα- and Gtγ-derived peptides are similar,[72] the mechanism of interaction between rhodopsin and the different binding sites on transducin, which leads to guanosine diphospate release, is not understood. Major future tasks in GPCR and rhodopsin research will therefore encompass determination of the structure of a GPCR·G-protein complex and detailed investigation of the process of its formation.

We thank Klaus Peter Hofmann and Kris Palczewski for critically reading the manuscript and Eglof Ritter for help with preparation of figures. Work in this laboratory has been supported by grants from the Deutsche Forschungsgemeinschaft.

[1] GPCRDB, *Information system for G-protein-coupled receptors (GPCRs)*. http://www.cmbi.kin.nl/7tm/.
[2] K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. Le Trong, D. C. Teller, T. Okada, R. E. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, *289*, 739 – 745.
[3] K. P. Hofmann, in *Handbook of Biological Physics, Vol. 3* (Eds.: D. G. Stavenga, W. J. DeGrip, E. N. Pugh, Jr.) Elsevier, Amsterdam, **2000**, pp. 91 – 142.
[4] T. P. Sakmar, *Prog. Nucleic Acid Res. Mol. Biol.* **1998**, *59*, 1 – 34.
[5] D. S. Kliger, J. W. Lewis, *Isr. J. Chem.* **1995**, *35*, 289 – 307.
[6] T. Okada, O. P. Ernst, K. Palczewski, K. P. Hofmann, *Trends Biochem. Sci.* **2001**, *26*, 318 – 324.
[7] S. T. Menon, M. Han, T. P. Sakmar, *Physiol. Rev.* **2001**, *81*, 1659 – 1688.
[8] Z. L. Lu, J. W. Saldanha, E. C. Hulme, *Trends Pharmacol. Sci.* **2002**, *23*, 140 – 146.
[9] D. Baylor, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 560 – 565.
[10] D. C. Teller, T. Okada, C. A. Behnke, K. Palczewski, R. E. Stenkamp, *Biochemistry* **2001**, *40*, 7761 – 7772.
[11] F. Siebert, *Isr. J. Chem.* **1995**, *35*, 309 – 323.
[12] J. W. Lewis, D. S. Kliger, *Methods Enzymol.* **2000**, *315*, 164 – 178.
[13] K. Fahmy, T. P. Sakmar, F. Siebert, *Methods Enzymol.* **2000**, *315*, 178 – 196.
[14] R. W. Schoenlein, L. A. Peteanu, R. A. Mathies, C. V. Shank, *Science* **1991**, *254*, 412 – 415.
[15] A. Cooper, *Nature* **1979**, *282*, 531 – 533.
[16] I. Palings, J. A. Pardoen, E. van den Berg, C. Winkel, J. Lugtenburg, R. A. Mathies, *Biochemistry* **1987**, *26*, 2544 – 2556.
[17] K. A. Bagley, V. Balogh-Nair, A. A. Croteau, G. Dollinger, T. G. Ebrey, L. Eisenstein, M. K. Hong, K. Nakanishi, J. Vittitow, *Biochemistry* **1985**, *24*, 6055 – 6071.
[18] F. Siebert, W. Mantele, K. Gerwert, *Eur. J. Biochem.* **1983**, *136*, 119 – 127.
[19] K. Fahmy, F. Jager, M. Beck, T. A. Zvyaga, T. P. Sakmar, F. Siebert, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10206 – 10210.
[20] U. M. Ganter, W. Gartner, F. Siebert, *Biochemistry* **1988**, *27*, 7480 – 7488.
[21] S. J. Hug, J. W. Lewis, C. M. Einterz, T. E. Thorgeirsson, D. S. Kliger, *Biochemistry* **1990**, *29*, 1475 – 1485.
[22] B. Borhan, M. L. Souto, H. Imai, Y. Shichida, K. Nakanishi, *Science* **2000**, *288*, 2209 – 2212.
[23] F. Jager, S. Jager, O. Krautle, N. Friedman, M. Sheves, K. P. Hofmann, F. Siebert, *Biochemistry* **1994**, *33*, 7389 – 7397.
[24] A. Akita, S. P. Tanis, M. Adams, V. Balogh-Nair, K. Nakanishi, *J. Am. Chem. Soc.* **1980**, *102*, 6370 – 6372.
[25] T. Zankel, H. Ok, R. Johnson, C. W. Chang, N. Sekiya, H. Naoki, K. Yoshihara, K. Nakanishi, *J. Am. Chem. Soc.* **1990**, *112*, 5387 – 5388.
[26] W. J. DeGrip, J. van Oostrum, P. H. Bovee-Geurts, R. van der Steen, L. J. van Amsterdam, M. Groesbeek, J. Lugtenburg, *Eur. J. Biochem.* **1990**, *191*, 211 – 220.
[27] S. Bhattacharya, K. D. Ridge, B. E. Knox, H. G. Khorana, *J. Biol. Chem.* **1992**, *267*, 6763 – 6769.
[28] G. F. Jang, V. Kuksa, S. Filipek, F. Bartl, E. Ritter, M. H. Gelb, K. P. Hofmann, K. Palczewski, *J. Biol. Chem.* **2001**, *276*, 26148 – 26153.
[29] F. Jager, K. Fahmy, T. P. Sakmar, F. Siebert, *Biochemistry* **1994**, *33*, 10878 – 10882.
[30] D. L. Farrens, C. Altenbach, K. Yang, W. L. Hubbell, H. G. Khorana, *Science* **1996**, *274*, 768 – 770.
[31] C. Altenbach, J. Klein-Seetharaman, K. Cai, H. G. Khorana, W. L. Hubbell, *Biochemistry* **2001**, *40*, 15493 – 15500.
[32] M. Gutman, E. Nachliel, *Biochim. Biophys. Acta* **1990**, *1015*, 391 – 414.
[33] C. K. Meyer, M. Bohme, A. Ockenfels, W. Gartner, K. P. Hofmann, O. P. Ernst, *J. Biol. Chem.* **2000**, *275*, 19713 – 19718.
[34] R. Vogel, G. B. Fan, M. Sheves, F. Siebert, *Biochemistry* **2000**, *39*, 8895 – 8908.
[35] R. Vogel, G. B. Fan, F. Siebert, M. Sheves, *Biochemistry* **2001**, *40*, 13342 – 13352.
[36] S. Arnis, K. P. Hofmann, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 7849 – 7853.
[37] S. Arnis, K. Fahmy, K. P. Hofmann, T. P. Sakmar, *J. Biol. Chem.* **1994**, *269*, 23879 – 23881.
[38] K. Fahmy, T. P. Sakmar, F. Siebert, *Biochemistry* **2000**, *39*, 10607 – 10612.
[39] A. Scheer, F. Fanelli, T. Costa, P. G. De Benedetti, S. Cotecchia, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 808 – 813.
[40] C. Seibert, C. Harteneck, O. P. Ernst, G. Schultz, K. P. Hofmann, *Eur. J. Biochem.* **1999**, *266*, 911 – 916.
[41] P. Ghanouni, H. Schambye, R. Seifert, T. W. Lee, S. G. Rasmussen, U. Gether, B. K. Kobilka, *J. Biol. Chem.* **2000**, *275*, 3121 – 3127.
[42] S. Nishimura, J. Sasaki, H. Kandori, J. Lugtenburg, A. Maeda, *Biochemistry* **1995**, *34*, 16758 – 16763.
[43] Y. J. Ohkita, J. Sasaki, A. Maeda, T. Yoshizawa, M. Groesbeek, P. Verdegem, J. Lugtenburg, *Biophys. Chem.* **1995**, *56*, 71 – 78.
[44] P. Rath, F. DeLange, W. J. DeGrip, K. J. Rothschild, *Biochem. J.* **1998**, *329*, 713 – 717.
[45] T. Nagata, A. Terakita, H. Kandori, D. Kojima, Y. Shichida, A. Maeda, *Biochemistry* **1997**, *36*, 6164 – 6170.
[46] T. Nagata, A. Terakita, H. Kandori, Y. Shichida, A. Maeda, *Biochemistry* **1998**, *37*, 17216 – 17222.
[47] T. Okada, Y. Fujiyoshi, M. Silow, J. Navarro, E. M. Landau, Y. Shichida, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5982 – 5987.
[48] T. J. Melia, Jr., C. W. Cowan, J. K. Angleson, T. G. Wensel, *Biophys. J.* **1997**, *73*, 3182 – 3191.
[49] R. Vogel, F. Siebert, *J. Biol. Chem.* **2001**, *276*, 38487 – 38493.
[50] P. R. Robinson, G. B. Cohen, E. A. Zhukovsky, D. D. Oprian, *Neuron* **1992**, *9*, 719 – 725.
[51] G. B. Cohen, T. Yang, P. R. Robinson, D. D. Oprian, *Biochemistry* **1993**, *32*, 6111 – 6115.
[52] M. Han, S. O. Smith, T. P. Sakmar, *Biochemistry* **1998**, *37*, 8253 – 8261.
[53] G. B. Cohen, D. D. Oprian, P. R. Robinson, *Biochemistry* **1992**, *31*, 12592 – 12601.
[54] S. Jager, K. Palczewski, K. P. Hofmann, *Biochemistry* **1996**, *35*, 2901 – 2908.
[55] K. Sachs, D. Maretzki, C. K. Meyer, K. P. Hofmann, *J. Biol. Chem.* **2000**, *275*, 6189 – 6194.
[56] S. Arnis, K. P. Hofmann, *Biochemistry* **1995**, *34*, 9333 – 9340.
[57] T. P. Williams, *Vision Res.* **1968**, *8*, 1457 – 1466.
[58] R. G. Matthews, R. Hubbard, P. K. Brown, G. Wald, *J. Gen. Physiol.* **1963**, *47*, 215 – 240.
[59] O. P. Ernst, C. K. Meyer, E. P. Marin, P. Henklein, W. Y. Fu, T. P. Sakmar, K. P. Hofmann, *J. Biol. Chem.* **2000**, *275*, 1937 – 1943.
[60] C. Grimm, C. E. Reme, P. O. Rol, T. P. Williams, *Invest. Ophthalmol. Visual Sci.* **2000**, *41*, 3984 – 3990.
[61] O. P. Ernst, C. Bieri, H. Vogel, K. P. Hofmann, *Methods Enzymol.* **2000**, *315*, 471 – 489.
[62] F. J. Bartl, E. Ritter, K. P. Hofmann, *J. Biol. Chem.* **2001**, *276*, 30161 – 30166.
[63] C. Grimm, A. Wenzel, T. Williams, P. Rol, F. Hafezi, C. Reme, *Invest. Ophthalmol. Visual Sci.* **2001**, *42*, 497 – 505.
[64] W. J. DeGrip, K. J. Rothschild, in *Handbook of Biological Physics, Vol. 3* (Eds.: D. G. Stavenga, W. J. DeGrip, E. N. Pugh, Jr.) Elsevier, Amsterdam, **2000**, pp. 1 – 54.
[65] D. Emeis, H. Kuhn, J. Reichert, K. P. Hofmann, *FEBS Lett.* **1982**, *143*, 29 – 34.
[66] H. E. Hamm, D. Deretic, A. Arendt, P. A. Hargrave, B. Koenig, K. P. Hofmann, *Science* **1988**, *241*, 832 – 835.
[67] O. G. Kisselev, M. V. Ermolaeva, N. Gautam, *J. Biol. Chem.* **1994**, *269*, 21399 – 21402.
[68] O. G. Kisselev, C. K. Meyer, M. Heck, O. P. Ernst, K. P. Hofmann, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 4898 – 4903.
[69] S. Nishimura, J. Sasaki, H. Kandori, T. Matsuda, Y. Fukada, A. Maeda, *Biochemistry* **1996**, *35*, 13267 – 13271.
[70] S. Nishimura, H. Kandori, A. Maeda, *Biochemistry* **1998**, *37*, 15816 – 15824.
[71] K. Fahmy, *Biophys. J.* **1998**, *75*, 1306 – 1318.
[72] F. Bartl, E. Ritter, K. P. Hofmann, *FEBS Lett.* **2000**, *473*, 259 – 264.
[73] T. Yoshizawa, Y. Shichida, *Methods Enzymol.* **1982**, *81*, 333 – 354.
[74] K. P. Hofmann, S. Jäger, O. P. Ernst, *Isr. J. Chem.* **1995**, *35*, 339 – 355.
[75] J. K. McBee, K. Palczewski, W. Baehr, D. R. Pepperberg, *Prog. Retinal Eye Res.* **2001**, *20*, 469 – 529.

Received: May 14, 2002 [M419]