Minireview

The eye photoreceptor protein rhodopsin. Structural implications for retinal disease[1]

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Received 19 July 2002; revised 8 August 2002; accepted 9 August 2002

First published online 22 August 2002

Edited by Vladimir Skulachev

Abstract Rhodopsin is the membrane receptor responsible for photoreception in the vertebrate retina. Its characteristic seven-transmembrane helical structural motif is today widely recognised as a paradigm in signal transduction. Rhodopsin and the phototransduction system are frequently used as structural and mechanistic models for the G-protein coupled receptor superfamily. Recent advances in the activation mechanism (as derived from the structural available data) and the implications for normal and pathological – in retinal disorders – visual function will be reviewed. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Rhodopsin; Retinitis pigmentosa; Congenital stationary night blindness; Misfolding; Constitutive activity

1. Introduction

Rhodopsin has captured researchers' attention since the isolation of *visual purple* by Kühne [1], and its early proposal as the key molecule with a central role in vision. In its pioneer studies on the molecular basis of vision, George Wald determined the chemical nature of the chromophoric light-absorbing molecule in rhodopsin. He found the antenna responsible for capturing a photon of visible light to be the 11-*cis* isomer of the derivative of vitamin A, retinal [2,3]. This system is widespread throughout the animal kingdom and the universality of 11-*cis*-retinal has been clearly established as more animal visual systems have been analysed [4]. An opsin protein and the chromophore 11-*cis*-retinal (or a slightly modified derivative) are virtually found in most animals eyes, whatever the species and evolutionary diversity. Retinal is a remarkable molecule very well adapted for its role as the biochemical initiator of the complex process of vision. The conservation of this molecule provides evidence of its importance in the physiological process in which it is involved. Nature has found, through evolution, an extremely effective molecular design, and it is much easier for the protein part of the pigment to change 'around' the chromophore. This allows spectral fine tuning of the different animal visual sensitivities to be achieved.

Rhodopsin consists of the apoprotein opsin – 348 amino acid residues – and the 11-*cis*-retinal chromophore covalently bound to Lys-296 in the seventh transmembrane helix, through a protonated Schiff base (PSB) linkage [5–8]. It belongs to the G-protein coupled receptor (GPCR) superfamily [9–13]. The members of this family share a basic seven-transmembrane helical design (Fig. 1), and their basic function involving binding and activating a G-protein, transducin in the case of the visual system [14–16]. It is the main protein component of the stacked disks found in the outer segments of rod cells (rod outer segments, ROS) in the vertebrate retina. It was found that rhodopsin comprises about 70% of the total protein in osmotically intact frog ROS [17], but this percentage increases to about 90–95% after the extensive washing steps used in the purification of ROS membranes. ROS membranes, obtained according to a number of slightly modified protocols from the sucrose gradient method [18], are highly enriched in rhodopsin. Although ROS are not homogeneously pure samples, they have been used for many structural and functional studies with the reasonable assumption that the obtained results were basically attributable to rhodopsin. Many of the studies carried out in the last decade using wild-type and mutant recombinant rhodopsins have dealt with purified rhodopsin. Purification of recombinant rhodopsin is necessary to separate rhodopsin from the many other proteins present in the mammalian cell culture systems where recombinant opsin is usually expressed [19,20]. The studies carried out by using ROS rhodopsin, either in membranes [21–23] or in detergent-solubilised systems [24,25], in combination with the wealth of information derived from biochemical and biophysical studies on recombinant mutant rhodopsins [26–32], have allowed an increasing body of knowledge about the structure of rhodopsin to be obtained. A recent landmark in rhodopsin structural studies has been the three-dimensional structural model obtained from the X-ray crystal structure at 2.8 Å resolution [33]. This study has been subsequently refined [34] and it has stimulated a renewed interest in the molecule. It has also inspired many scientific contributions, ranging from commentaries [16,35,36] to a number of studies dealing with other members of the GPCR superfamily [37–39]. This has strengthened the role of rhodop-

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[1] This paper is dedicated by P.G. to Prof. H. Gobind Khorana on the occasion of his 80th birthday.

Abbreviations: GPCR, G-protein coupled receptor; ROS, rod outer segments; PSB, protonated Schiff base; MetaII, metarhodopsin II; RP, retinitis pigmentosa; CSNB, congenital stationary night blindness

0014-5793/02/$22.00
PII: S0014-5793(02)03241-6

sin as the model of this superfamily, at least for the so-called rhodopsin-like subfamily since the possible extrapolation of rhodopsin features to the whole superfamily may not be as straightforward [36].

2. Rhodopsin and the mechanism of phototransduction activation

Rhodopsin responds to the electromagnetic radiation by means of the retinal chromophore. Upon photon capture retinal isomerises through its 11–12 C–C bond to its all-*trans* configuration. As a result of this primary photochemical event, which is one of the fastest reactions in nature (taking place in the order of fs), opsin evolves through a number of thermal intermediates which cannot be detected at room temperature. This leads to the formation of the active conformation metarhodopsin II (MetaII), which has retinal still covalently linked to opsin. This response of the protein to the primary physicochemical isomerisation of 11-*cis*-retinal includes a number of changes in the protonation state of ionisable residue side chains critical in the activation process. The first of these changes is deprotonation of the Schiff base nitrogen occurring as a result of the pK_a change after the conformational rearrangement ensuing retinal isomerisation. This is accompanied by protonation of the Glu-113 counterion, which has been proposed as the Schiff base proton acceptor [40]. The disruption of the salt bridge between ionised Glu-113 and the PSB nitrogen, present in native dark rhodopsin, is today clearly recognised as one of the critical switches for the active conformation to be achieved. The presence of this salt bridge interaction in ground state rhodopsin is clearly a main stabilising feature of the inactive conformation of the receptor. More recent data reveal a more complex picture where a number of other intramolecular interactions, both of an electrostatic but also of an steric nature, are involved in the complete stabilisation of the inactive conformation of the receptor. This means that several factors, other than breakage of the Glu-113/Schiff base ionic interactions, are required for the fully activated rhodopsin to be obtained [16,41,42]. Among these the first one to be observed was Glu-134 protonation. This step is clearly established as necessary for the formation of activated rhodopsin and has been related to the distinction of the two variants of the MetaII conformation called MetaIIa and MetaIIb [43].

Transducin activation by MetaII initiates the visual transduction pathway, which results in hyperpolarisation of the rod cell membrane that eventually generates a nervous signal to the brain. A number of genes encoding proteins participating in this cascade of biochemical reactions, but also in photoreceptor structure and others, have been associated with retinal diseases, particularly retinitis pigmentosa (RP) [44]. Mutations in many of these genes have been clearly determined to be the cause of these diseases. In particular, mutations in rhodopsin account for about 25% of all cases of the autosomic form of RP and are also associated with congenital stationary night blindness (CSNB) [45].

3. Rhodopsin mutations and retinal disease

Rhodopsin was the first visual protein where a point mutation was found to cause a pathological hereditary condition, and the mutation cosegregated with the retinal disease in an affected family. This mutation in the opsin gene produced a Pro23His substitution [46,47] in the intradiscal domain of the protein, and was a founder mutation in the North American population.

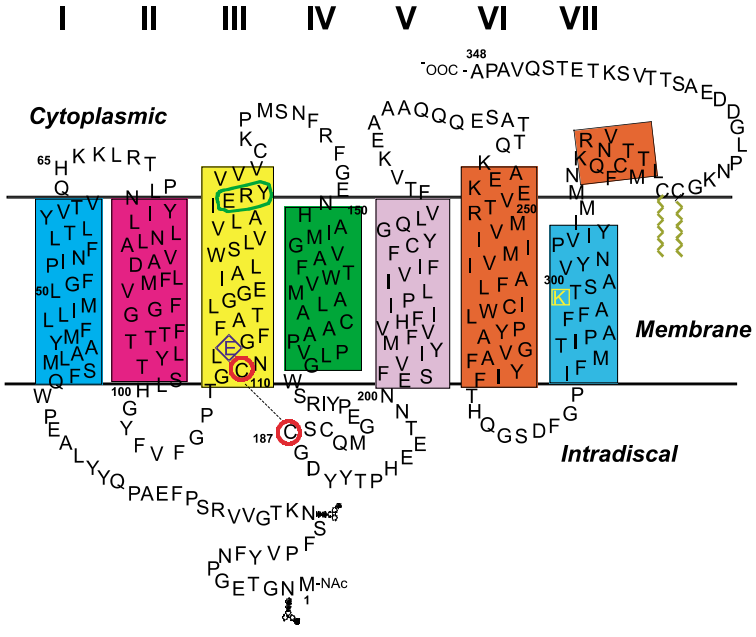

Fig. 1. Secondary structure model of rhodopsin based on the crystal structure data at 2.8 Å [33]. Several distinctive features are shown: (i) in transmembrane helix III, Glu-113 (in blue), the counterion to the PSB; Cys-110, one of the two cysteine residues involved in the highly conserved disulfide bond with Cys-187 in the second intradiscal loop (in red circles); the ERY conserved triplet (circled in green) at the cytoplasmic side of the protein very important for transducin activation in the visual phototransduction cascade; (ii) in transmembrane helix VII, the site of 11-*cis*-retinal attachment, Lys-296 (in yellow). Also shown are the palmitoylated Cys-322 and Cys-323 which form the putative fourth loop by anchoring in the phospholipid membrane and providing the constraint for the eighth helix in the cytoplasmic domain, and glycosylation at Asn-2 and Asn-15.

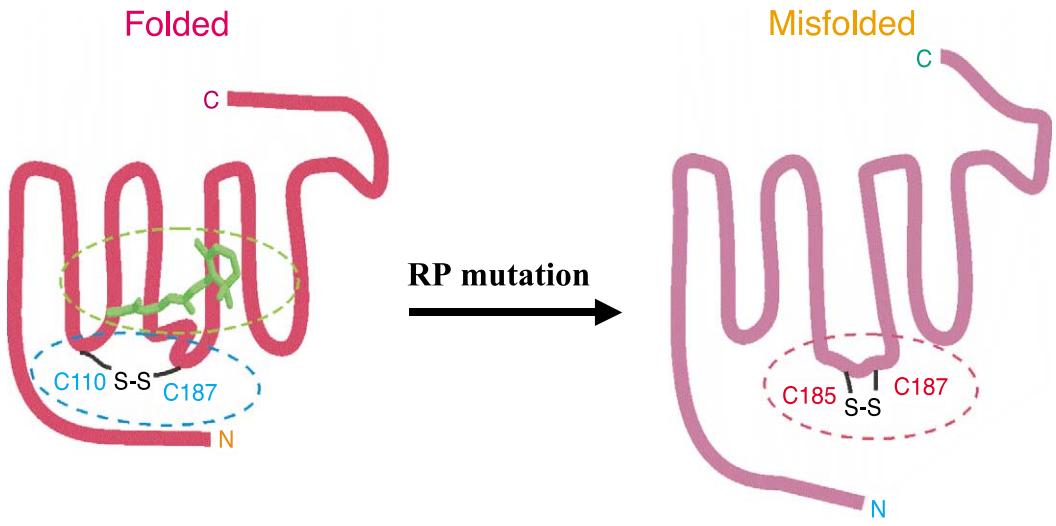

Fig. 2. Schematic representation of the main consequence of mutations associated with RP in rhodopsin at the structural level. The folded conformation in the dark has the right configuration of the retinal binding pocket and it allows efficient finding of this ligand by maintaining the correct set of interactions. The RP mutation results in an altered conformation of the protein which would disrupt the compact tertiary structure in the intradiscal domain. This would, in turn, allow the formation of a wrong disulfide bond between Cys-185 and Cys-187 that would irreversibly lock the protein in its misfolded conformation. One of the consequences would be that the correct binding pocket of the retinal is not formed and 11-*cis*-retinal binding is, therefore, either abolished or severely impaired. Other structural effects of this misfolding may be changes in the helices and in the cytoplasmic loops and C-terminal tail as well.

The study of the molecular mechanism underlying retinal degeneration caused by mutations in retinal proteins, and particularly in rhodopsin, is a matter of interest for the future development of suitable therapies. The accessibility of the retina, which can be scrutinised without using invasive techniques, makes it a part of the neurological system amenable to be easily studied at the clinical level (the retina has been called *the approachable part of the brain*). This interest is not exclusive to the neuroscience field but it is also very relevant to the elucidation of the molecular basis of disease, in particular of those diseases associated with mutations in members of the GPCR superfamily. In this regard, correlation of the molecular alteration in the membrane protein rhodopsin caused by a point mutation, together with its cellular consequences, with the clinical phenotype of the retinal disease in RP patients is one of the goals that remain unattained. Although several potential mechanisms for the degenerative process in RP have been proposed, a clear detailed explanation, other than retinal cell death may go through an apoptotic pathway [48,49], has not been provided yet. Several aspects related to retinal diseases caused by mutations in rhodopsin are still a puzzle to scientists involved in vision research; among them: (i) rhodopsin mutations occurring in rod cells result also in cone cell death; (ii) very similar mutations in rhodopsin can cause different clinical phenotypes like those associated with RP and CSNB; (iii) the same mutation has very different consequences, in individuals of the same family, on the onset and severity of the RP condition. While the first question remains unanswered, the third question seems to obviously point to the involvement of other factors, other than the mutation, in the physiopathology of RP. Concerning the difference between RP and CSNB there is increasing evidence that these two conditions may have different molecular mechanisms as will be discussed below.

Recent studies suggest interesting novel molecular mechanisms for RP, like the one recently proposed where rod cell death could result from activation of mislocalised opsin [50]. Another proposal has been made suggesting that spreading of

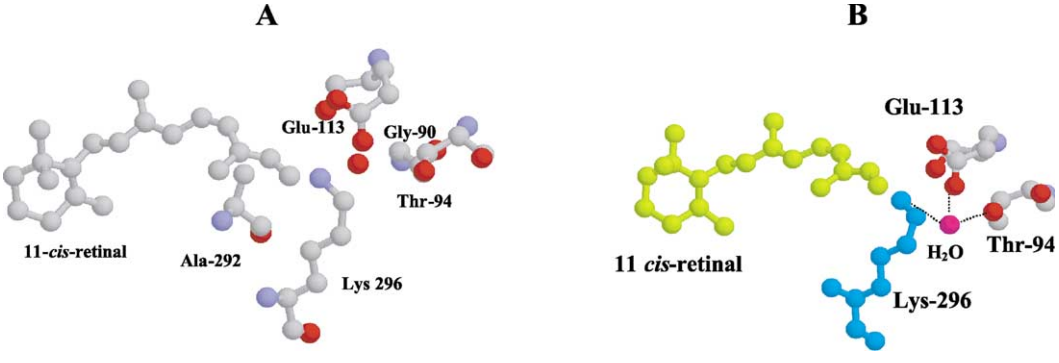

Fig. 3. A: Amino acid side chain arrangement of the residues mutated in CSNB, namely Gly-90, Ala-292 and Thr-94, in the vicinity of the PSB and close to the Glu-113 counterion. B: Location of the water molecule proposed to be in this region [65] in proximity to Glu-113 and Thr-94. Disruption of the network of electrostatic interactions affecting these residues could allow a change in the pK_a of the PSB. This would be in the basis of the molecular mechanism underlying CSNB (see text).

photoreceptor cell death from rods to cones may be caused by gap junctions that exist between these photoreceptor subtypes [51].

4. Mutations in rhodopsin associated with RP

Up to 150 mutations have been reported to date in the opsin gene associated with the retinal degenerative RP since the Pro23His mutation, in the intradiscal domain of the protein, was first reported in 1990 [44]. Most of them are related with the autosomical form of the disease (adRP) and are single point missense mutations. These mutations are found widespread throughout the opsin gene and they are distributed along the three domains of the protein, the intradiscal, transmembrane and cytoplasmic domains. This plethora of mutations can be regarded as 'natural hints' of residues in the polypeptide opsin chain important for its optimal structure and/or function. The large number of mutations found and their different location may be indicative of a highly sophisticated arrangement of physicochemical interactions in the three-dimensional structure of the native rhodopsin molecule. This has been naturally designed to account for an optimised protein system that can control several very important physiological aspects of the complex phenomenon of vision. Among these, we can highlight the precise set of amino acid side chain interactions with the retinal chromophore in the retinal binding pocket to allow fine tuning of the different visual pigments. Also, the dark conformation of the receptor has an extraordinary stability, with dark noise resulting from thermal activation of the receptor being very low to allow adaptation to very low light intensity levels to be achieved. At the same time the system is also extremely efficient in the activation of the phototransduction cascade and in the amplification of the system. The activation and inactivation processes in which rhodopsin is involved, in the visual biochemical cycle, are precisely modulated by a number of very well organised sets of protein–protein interactions where metal ions play also an important role. The details of these interactions are currently being unravelled and there is a growing body of evidence that supports the view of a complex but very well designed biochemical system. With this outlined picture it is easily understood that rhodopsin mutations responsible for RP must be altering this precise network of interactions as a consequence of the different degrees of perturbation of the rhodopsin structure. Obviously, modifications in the native conformation of rhodopsin as a result of RP mutation can result in altered function, i.e. alterations in transducin activation. In the past decade several research groups have devoted sustained effort to elucidate the molecular mechanism(s) underlying the retinal RP pathology. The ultimate goal of this research is to contribute to the development of therapeutic solutions to the disease. Several mechanisms have been outlined but there is no clear explanation about the molecular link between the rhodopsin mutation and the rod cell degenerative process. From a biochemical standpoint it is undoubtedly of interest to obtain detailed knowledge of the phenotype of the mutant proteins and to correlate the molecular defect observed with the clinical phenotype of the affected RP patients. This clinical correlation with the laboratory results has turned out to be not straightforward due to the extreme clinical and genetic heterogeneity of the disease. The onset and the progression of the disease have been found to be very different in relatives carrying the same opsin mutation. Besides, there are some RP mutations that apparently do not alter significantly the structure or function of the protein, so it is not clear which might be the cause of the disease in these cases. In spite of these problems, it is interesting to try to establish a correlation between protein structural features and RP patient clinical phenotype taking into account the different current hypothesis of how these two aspects may be linked.

Protein misfolding has been proposed to be one of the main biochemical causes of RP in the case of rhodopsin mutations [52–54], and molecular chaperones have been also involved in RP [55]. At the molecular level non-native disulfide bond formation between Cys-185 and Cys-187 has been recently shown in misfolded opsin (non-retinal binding) fractions by mass spectrometry [56] (Fig. 2). Misfolding seems to be a general theme in many of the diseases caused by mutations, and particularly with those associated with rhodopsin in RP. Altered stability and/or function may also have relevance for a number of given mutations. In this regard, abnormal functioning resulting in reduced activity or hyperactivity could also play a role in RP by altering the stoichiometric balance of the different proteins involved in the phototransduction biochemical reactions. It is important to note that hyperactivity, in the case of RP, is not necessarily related to constitutive activity (that is activity in the absence of ligand or light), but to increased initial activation rate in some mutants (A. Andres, E. Roca, P. Garriga and J. Manyosa, to be submitted). Some mutations, like those in the C-terminal tail, might affect post-Golgi trafficking of rhodopsin and may result in mislocalised rhodopsin [57]. In this case the aforementioned mechanism of rod cell death triggered by mislocalised opsin [50] would provide an explanation for the biochemical basis of RP related to this kind of mutations. This may be the case for several mutations, like Gln344ter and Pro347Leu, known to have a clinical severe phenotype, located in the C-terminal sequence QVS(A)PA which has been proposed to comprise a signal recognisable by factors in the trans-Golgi network [58].

5. Mutations in rhodopsin associated with CSNB

CSNB is another retinal disease also associated with rhodopsin mutations. In contrast with RP, only three mutations have been found associated with CSNB in rhodopsin. It is possible that the more benign phenotype of this pathology has prevented more mutations to be discovered. Two of these mutations are found in the second transmembrane helix of rhodopsin at Gly-90 and Thr-94. These correspond to Gly90Asp [59] and Thr94Ile, which has been the latest one to be reported [60]. The other mutation, Ala292Glu [61] is located in the seventh transmembrane helix, in proximity to the site of retinal attachment at Lys-296. The three residues are located in the environment of the PSB and the Glu-113 counterion (Fig. 3). The electrostatic interaction between the Glu-113 counterion and the PSB nitrogen is known to be one of the key features in maintaining the inactive conformation of the receptor. Relieving this constraint by breakage of the salt bridge has been proposed to be one of the requirements for the active conformation to be achieved [62]. In the case of the Gly90Asp mutation a molecular mechanism was proposed for CSNB on the basis of constitutive activation of the mutant receptor [59]. This mutation has been analysed in some detail

[63] and the mechanism of constitutive light adaptation, without rod cell loss, has been recently confirmed in transgenic mice carrying the Gly90Asp mutation [64]. The same mechanism was also proposed for the Ala292Glu mutation [62]. In the two cases, the proposed mechanism involves partial disruption of the native salt bridge, by electrostatic competition of the introduced residue side chain with Glu-113 for the PSB nitrogen. According to this molecular model the resulting constitutive activity in the dark, induced by the structural changes caused by the mutation, would be in the basis of the physiological effects seen in CSNB. In addition to its importance as a visual pathology, the study of CSNB is interesting because it can be a model of the first stages of RP. The molecular mechanism of the novel mutation found in transmembrane helix II, Thr94Ile, does not appear to fit in the 'electrostatic model' outlined above. In this particular case, the hydrophobic and bulkier isoleucine side chain replaces the polar hydrophilic threonine side chain, and there is no possibility of electrostatic competition as proposed for the other two CSNB related mutants. The The94Ile mutant shows highly differentiated features when compared to other mutants, namely very high thermal instability, high hydroxylamine reactivity and an extremely long-lived MetaII species (E. Ramon, L. del Valle and P. Garriga, to be submitted). Constitutive activity of this mutant, although presumed, has not been established yet and it may be difficult to determine possibly because of stability problems of the protein. Thr-94 is located in helix II one helix turn apart of Gly-90 and seen to be in close proximity to Glu-113 in the three-dimensional model of rhodopsin derived from the crystal structure at 2.8 Å resolution [33]. Recently the location of water molecules and its role in the activation mechanism of rhodopsin has been reported [65]. One of the water molecules would be located in proximity to the 90–94 region because it has been proposed to be in the vicinity of Phe-91 [65]. It is possible that Thr-94 is interacting with Glu-113 directly or through a water molecule. Replacement by isoleucine would eliminate this interaction, and this could result in a lowering of the pK_a of the PSB with a similar outcome to that caused by the other two mutations. Thus, the emerging picture is more complex than previously thought and may involve multi-part interactions in the vicinity of the PSB. With this vision in mind, the three CSNB mutations would be acting by means of a mechanism involving changes in electrostatic interactions and subsequent lowering of the pK_a for the PSB, thus facilitating its deprotonation.

Taking into account all the information known to date, it is likely that the molecular defect in rhodopsin underlying RP is related to partial or complete misfolding and the resulting inability of the mutant proteins to bind 11-*cis*-retinal. In contrast, mutations associated with CSNB affect amino acid residues that cluster around the PSB linkage and are presumably associated with changes in conformational stability and the protonated status of the PSB nitrogen. In this latter case, the derived functional alterations of the mutant receptors would be in the basis of the observed phenotypes in CSNB, rather than gross structural misfolding as associated with RP mutations.

Acknowledgements: We would like to thank the members of the authors' laboratories for helpful discussions, especially Anna Andrés, Eva Ramon, Luis del Valle and Laia Bosch. The work in the authors' laboratories has been supported by grants PM98-0134 (to P.G.) and PB98-0936-C03-02 (to J.M.) from DGESIC, and from FUNDALUCE and ONCE (to P.G. and J.M.).

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