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Altered functionality in rhodopsin point mutants associated with retinitis pigmentosa ☆

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Abstract

Point mutations found in rhodopsin associated with the retinal degenerative disease retinitis pigmentosa have been expressed in mammalian COS-1 cells, purified, and characterised. The mutations characterised—most of them for the first time—have been Met44Thr, Gly114Asp, Arg135Leu, Val137Met, and Pro171Leu in the transmembrane domain; Leu328Pro and Ala346Pro in the C-terminal tail of the cytoplasmic domain; and Gly106Trp in the intradiscal domain. Several of these mutations cause misfolding which results in impaired 11-*cis*-retinal binding. Two of them, Met44Thr and Val137Met, show spectral and structural features similar to those of wild type rhodopsin (Type I mutants) but significantly increased transducin initial activation rates. We propose that, in the case of these mutants, abnormal functioning resulting in faster activation kinetics could also play a role in retinitis pigmentosa by altering the stoichiometric balance of the different proteins involved in the phototransduction biochemical reactions.

Keywords: G-protein-coupled receptor; Protein misfolding; Transducin activation; Retinitis pigmentosa

Retinitis pigmentosa (RP) is a family of ocular diseases causing retinal degeneration with an important clinical and genetic heterogeneity [1,2]. Symptoms include progressive night blindness, rod cell degeneration accompanied by cone loss, and decrease in electroretinogram potentials [3]. This degenerative process eventually results in the loss of overall retinal function. RP can be inherited in several forms, as an X-linked trait, autosomal recessive, autosomal dominant or sporadic case [4]. An increasing number of genes directly associated (or predicted to be) with RP are being reported, together with many more loci for allied diseases and syndromic forms [1,5]. Rhodopsin mutations are the most common cause for autosomic dominant retinitis pigmentosa, and they are responsible for about 25% of autosomic dominant retinitis pigmentosa and some cases of autosomic recessive retinitis pigmentosa. To date, up to 150 mutations in the rhodopsin gene have been discovered [1].

Rhodopsin is the photoreceptor molecule from retinal rod cells [6–8]. This protein mediates scotopic vision and belongs to the G-protein-coupled receptor (GPCR) superfamily, whose structural distinctive feature is the basic heptahelical transmembrane motif [9–12]. The protein comprises three topological domains: the extracellular surface, the membrane-embedded domain, and the intracellular surface. The crystal structure of rhodopsin was recently resolved at 2.8 Å [13] and subsequently refined [14,15]. The helical segments form a compact bundle that contains the binding site for the chromophore 11-*cis*-retinal, covalently bound to Lys-296 in transmembrane helix VII by a protonated Schiff-base linkage, which is stabilised by the negatively charged counterion Glu-113 in transmembrane helix III. Disruption of this salt bridge [16,17] upon proton transfer is thought to trigger conformational changes in

☆ *Abbreviations:* RP, retinitis pigmentosa; GPCR, G-protein-coupled receptor; DM, *n*-dodecyl β-D-maltoside; UV–vis, ultraviolet-visible; GTPγS, guanosine 5′-O-(thiotriphosphate); wt, wild type.

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rhodopsin [18,19], which are necessary for transducin activation [20]. One of the interesting features of the retinal binding pocket is the presence of many polar or polarizable groups surrounding the hydrophobic chain of the retinal chromophore. Several amino acid side chains surround the imine moiety, including Tyr-43, Met-44, and Leu-47 in helix I, Thr-94 in helix II, and Phe-293 and Phe-294, helping in the correct positioning of the Lys-296 side chain [13,14].

In the transmembrane domain, and at the cytoplasmic end of helix III, the conserved ERY triplet (DRY in the GPCR superfamily), that has the critical Glu-134 residue involved in transducin activation [21], can be found. The importance of Glu-134 and Arg-135 for transducin activation has been demonstrated by means of site-directed mutagenesis that abolished this process [22–24]. A hydrophobic patch formed by Val-137/Val-138/Val-139 is also closely located and partly covers the cytoplasmic side of Glu-134 and Arg-135 [13].

In the present study we report on the results from the characterisation of some mutations in the opsin gene in Spanish patients affected by the autosomal form of RP. These mutations are Met44Thr, Gly114Asp, Arg-135Leu, Val137Met, Pro171Gln, and His211Arg in the transmembrane domain, Gly106Trp in the intradiscal domain, and Leu328Pro and Ala346Pro in the cytoplasmic domain (Fig. 1). The results obtained include reduced or no regeneration in several mutants, reduced thermal stability and reaction to hydroxylamine increased in the dark, and lower or higher ability (depending on the mutant) to activate transducin related to the wild type (wt) protein. We propose that, in the case of certain mutations (like those classified as Type I mutants associated with RP [25,26]), increased transducin activation initial rates may also be reported on the basis of a possible molecular mechanism of RP, in addition to structural misfolding proposed for some synthetic mutations [27] and many of the mutations associated with this retinal degenerative disease [28,29].

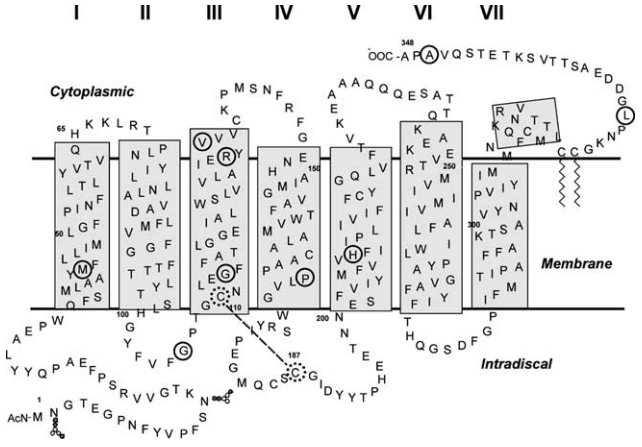

Fig. 1. Secondary structure model of rhodopsin where the amino acids mutated are shown in circles. These amino acids have been identified in retinitis pigmentosa patients and the mutations correspond to: Met44Thr in helix I; Gly106Trp in the extracellular loop E-I; Gly114Asp, Arg135Leu, and Val137Met in helix III; Pro171His is helix IV; His211Leu in helix V; and Leu328Pro and Ala346Pro in the C-terminal tail. The two cysteines involved in the conserved disulphide bond in the extracellular domain are shown in broken circles.

Materials and methods

Materials. 11-*cis*-retinal was provided by Dr. P. Philippov (Moscow State University). *n*-Dodecyl β-D-maltoside (DM) was from Anatrace (Maumee, OH, USA). Anti-rhodopsin monoclonal antibody rho-1D4 was obtained from the Cell Culture Center (National Institutes of Health, Bethesda, MD, USA) and was coupled to a cyanogen bromide-activated Sepharose 4B (Pharmacia). The buffers used are as follows: buffer A, sodium phosphate buffer (10 mM, pH 7.2) containing 2.7 mM KCl and 137 mM NaCl; buffer B, buffer A plus 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 1% (w/v) DM; buffer C, buffer A plus 0.05% DM. Elution of the proteins from the rho-1D4-Sepharose resin was carried out with buffer C containing 100 μM carboxyl-terminal (C')-nonapeptide. Hydroxylamine hydrochloride was purchased from Fluka Chemika.

Construction of mutant opsin genes. Rhodopsin mutants were obtained by site-directed mutagenesis in the synthetic opsin gene [30] cloned into the pMT4 vector [31]. The construction of the point-mutant genes containing Gly-114 → Asp, Pro-171 → Gln, His-211 → Arg, Leu-328 → Pro, and Ala-346 → Pro substitutions was performed using *cassette mutagenesis* in the synthetic opsin gene. This technique involved replacement of a restriction fragment in the synthetic opsin gene by a synthetic DNA duplex containing the appropriate codon change. For Gly-114 → Asp point mutant, the corresponding *Xho*I/*Nhe*I (3889 bp) and *Pvu*I/*Nhe*I fragments (2229 bp), in the pMT4 vector, were ligated with the annealed synthetic DNA duplex. Pro-171 → Gln and His-211 → Arg point mutants were generated by ligation of *Pml*I/*Bss*HII (3376 bp), *Pml*I/*Sfi*I (997 bp), and *Xba*I/*Bss*HII (1778 bp) fragments and *Eco*RI/*Not*I (5128 bp), *Ava*II/*Pvu*II (632, 374 bp) fragments, respectively, with the annealed synthetic DNA duplex. The Leu-328 → Pro mutant was obtained by ligation of *Sal*I/*Nhe*I (4506 bp) and *Bst*EII/*Nhe*I (1628 bp) fragments with the annealed synthetic oligonucleotides carrying the point mutation, and Pro-346 → Leu mutant was generated by ligation of *Sal*I/*Nhe*I (4554 bp) and *Not*I/*Nhe*I (1578 bp) fragments with the corresponding DNA duplex. Point mutants Met-44 → Thr, Gly-106 → Trp, Arg-135 → Leu, and Val-137 → Met were obtained using the kit QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). A polymerase chain reaction was performed using *Pfu* polymerase, pMT4-opsin as template, and oligonucleotide primers containing each mutation. Sense primers used for generation of Met-44 → Thr and Gly-106 → Trp mutants were, respectively, 5′-G**C**C**G**C**C**TA**C**A**C**GTT**C**CTG**C**TGAT**C**-3′ and 5′-CA**T** GGGTAC **TT**CGT**C**T**TTT**GGCCGACGGGC**T**GCAACC-3′; and for generation of Arg-135 → Leu and Val-137 → Met, 5′-GGCGA**T**CGAG C**T**GA**T**CG**T**GG**T**GG-3′ and 5′-CG**A**GCGGT**A**C**A**TGGTGGTGTG C-3′, respectively. Changes are indicated in bold. After amplification, the product was treated with *Dpn*I, which digests the parenteral construct. Finally, the nicked plasmids were transformed. The correct sequences for the mutant opsin genes were confirmed by DNA sequencing with Alfexpress Amersham–Pharmacia Biotech automatic sequencer.

Expression and purification of wt and mutant rhodopsins. The wt and the mutant genes were expressed in transiently transfected monkey kidney COS-1 cells, following the DEAE-dextran procedure, as previously described [32]. Purification of the wt and mutants with the rho-1D4 monoclonal antibody, after regeneration with 11-*cis*-retinal, was carried out as described [33]. The protein was finally eluted from the chromatographic column in buffer C. Electrophoretic analysis was

carried out by SDS/PAGE [34] and the proteins were electrotransferred onto nitrocellulose [35] and detected using the rho-1D4 monoclonal antibody in the Western blot analysis.

UV–visible absorption spectroscopic assays of wt and mutant pigments. UV–visible (UV–vis) absorption spectra of the purified proteins were obtained with a Cary 3 spectrophotometer (Varian, Australia) equipped with thermostatted cuvette holders. Spectra were recorded with a scan speed of 240 nm/min. The acquisition parameters used were 2 nm bandwidth and a response time of 0.1 s. All spectra were recorded at 20 °C except for the thermal bleaching experiments. The photobleaching and acidification behaviour assays of the wt and the mutants were carried out as described [33]. All the other different spectroscopic assays carried out in the visible region, i.e., thermal bleaching and hydroxylamine reactivity, were also carried out essentially as previously described [33].

Transducin activation by the mutant rhodopsins. Transducin was isolated from bovine rod outer segments as described [36] and transducin activation was monitored by fluorescence spectroscopy. The fluorescence assay measuring $G_\alpha \cdot GTP\gamma S$ (complex between α subunit of transducin and GTPγS) formation rate, catalysed by wt rhodopsin and mutant pigments upon illumination, was essentially carried out with a SLM Aminco 8100 fluorimeter as described previously [33]. Briefly, rhodopsin (40 nM) was added to a continuously stirred solution of transducin (250 nM) and GTPγS (5 μM) in 10 mM Tris–HCl, pH 7.4, 100 mM NaCl, 2 mM $MgCl_2$, and 0.012% DM. The reaction was initiated by illumination for 30 s with a 150 W-fibre optic light source through a 495-nm cut-off filter. The assay was carried out at a temperature of 20 °C. To calculate the relative activation rates, the slopes of the initial fluorescence increase after illumination were determined by linear regression of the data points corresponding to the first 60 s. The values for the mutants were normalised to the value obtained for wt rhodopsin taken as 1.00. The assay was carried out in DM solution as previously reported [26,29,33] and the significance of the results obtained was carefully analysed with regard to the molar concentrations of the mutant and wt samples (rhodopsin concentration was determined spectroscopically by using a ε_{500} of 40,600 $M^{-1}\,cm^{-1}$).

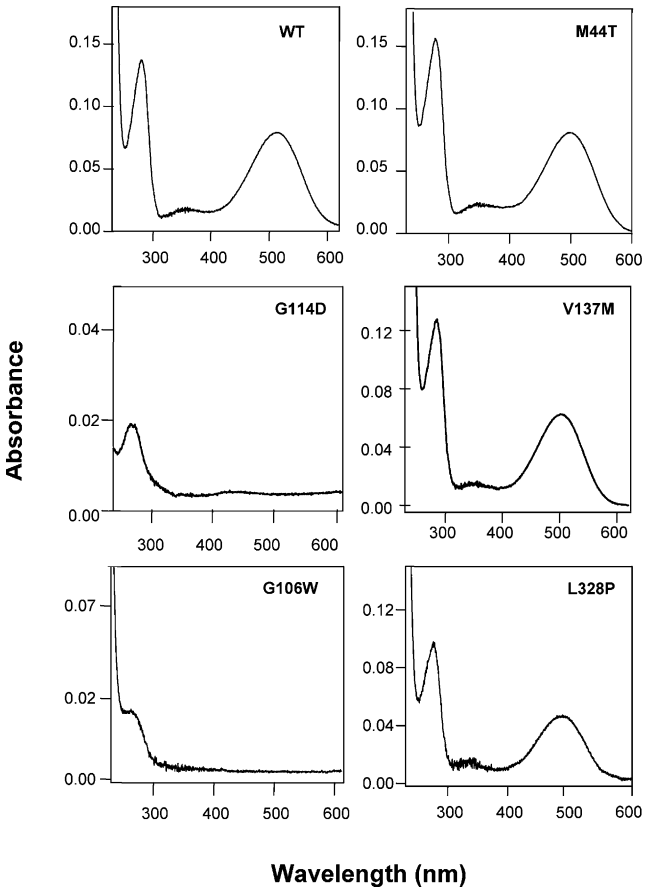

Fig. 2. Uv–vis absorption spectra of some of the purified mutant proteins and the wt after elution from the immunopurification process. The proteins are in sodium phosphate buffer (10 mM, pH 7.2) containing 2.7 mM KCl and 137 mM NaCl and 0.05% DM (buffer C). Temperature, 20 °C.

Results

Spectroscopic and electrophoretic analysis

Mutants in amino acid residues located in the transmembrane domain of rhodopsin in helix I (Met44Trh), helix III (Gly114Asp), helix IV (Pro171Gln), and helix V (His211Pro) were expressed in COS-1 cells and immunopurified by means of the rho-1D4 monoclonal antibody as described under Materials and methods. UV–vis absorption spectra of the purified mutants indicate that all of them, except for Met44Thr, fail to form chromophore with 11-*cis*-retinal (Fig. 2). The amount of protein recovered from the immunoaffinity column is very small—except for the Met44Thr mutant—when compared to that of the wt in spite of the fact that the same number of COS-1 cell plates was used in each case. The absence of chromophore regeneration for these mutants was confirmed when the mutants were photobleached, after incubation with 11-*cis*-retinal but before purification, and no difference spectra in the visible region could be detected (data not shown). In the case of Met44Thr, this mutant is able to form chromophore at a similar level to that of wt rhodopsin (Fig. 2) and the λ_{max} of the visible absorption band is located at 497 nm, slightly blue-shifted with regard to that of the wt rhodopsin. The electrophoretic pattern observed for this mutant, in the Western blot analysis, is the same as wt rhodopsin (Fig. 3A).

Mutants Arg135Leu and Val137Met located at the cytoplasmic boundary of helix III were expressed and regenerated chromophore. However, some differences could be detected between these two mutants. The UV–vis spectrum of Val137Met showed the characteristic visible band at 500 nm (Fig. 2) and an A_{280}/A_{500} of 1.8 very similar to that of the wt rhodopsin. In the case of Arg135Leu the chromophoric band was also located at 500 nm but the A_{280}/A_{500} ratio was slightly higher being about 2.5 (Table 1). These mutants show the same electrophoretic pattern as wt rhodopsin, but the expression of the Arg135Leu mutant was lower (Fig. 3B).

UV–vis spectrum of purified intradiscal Gly106Trp mutant shows the characteristic of UV band at 280 nm but no band at 500 nm, indicating that this mutant is unable to bind 11-*cis*-retinal. This mutant is expressed at very low levels when compared to the wt protein. The

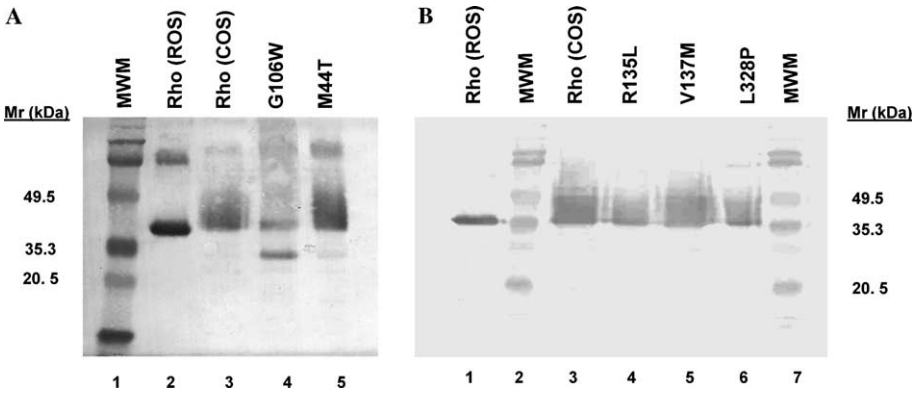

Fig. 3. Western blot analysis of the purified recombinant mutant and wt rhodopsins using the rho-1D4 monoclonal antibody. (A) 1, molecular weight marker; 2, rhodopsin from rod outer segments obtained from bovine retinas; 3, wt rhodopsin expressed in COS-1 cells; 4, Gly106Trp purified mutant; and 5, Met44Thr purified mutant. (B) 1, rhodopsin from rod outer segments; 2, molecular weight marker; 3, wt rhodopsin expressed in COS-1 cells; 4, Arg135Leu mutant; 5, Val137Met mutant; 6, Leu328Pro mutant; and 7, molecular weight marker.

Table 1
Summary of the results for the different mutants that regenerated with 11-*cis*-retinal

	λmax (nm)	A_{280}/A_{500}	Hydroxylamine reactivity ($k \cdot 10^5\ min^{-1}$)	Thermal stability ($t_{1/2}$, min)	Fluorescence transducin activation (initial rates)
WT	500	1.8	1.74	12.0	1.00
M44T	497	1.9	2.00	11.7	1.60
R135L	500	2.5	3.20	4.1	0.50
V137M	500	2.0	2.23	10.2	1.25
L328P	500	2.1	1.93	9.7	1.10
A346P	500	ND*	ND	ND	ND

In the first two columns the spectroscopic properties λmax of the visible band and the A_{280}/A_{500} ratio are presented. The hydroxylamine reactivity and the thermal stability values have been derived from the corresponding experimental curves obtained as described under Materials and methods. The initial activation rates have been normalised to the wilt type value taken as 1.00. The different values were obtained from at least three independent experiments and the maximal error observed was 10%. *ND, not determined because this mutant could not be purified with the rho-1D4 antibody column (the λmax of this mutant was estimated from the difference spectra of detergent solubilised COS-1 cells).

electrophoretic behaviour of this mutant is abnormal, showing the presence of a lower band at about 28 kDa below the main opsin band at 40 kDa (Fig. 3A).

The two mutants in the C-terminal tail of rhodopsin, Leu 328Pro and Ala346Pro, were expressed in the COS-1 cell system but the Ala346Pro could not be purified with the rho-1D4 antibody most likely because it is located at the recognition epitope for the antibody. In the case of the Leu328Pro mutant it was expressed normally and the UV–vis spectrum showed the 500-nm band but a slightly higher A_{280}/A_{500} of 2.1. The expression of the Ala346Pro mutant and its chromophore regeneration was verified by photobleaching difference spectra on COS cell membranes expressing the mutant after reconstitution and before purification (data not shown). These spectra indicated that Ala346Pro mutant was expressed and regenerated to a similar level to that of the wt protein.

Bleaching and acidification behaviour

The wt showed the characteristic shift of the chromophoric visible band from 500 to 380 nm upon illumination for 10 s with light > 495 nm. Subsequent acidification resulted in reprotonation of the Schiff base linkage at the visible band shifted back to 440 nm (Fig. 4) as expected. All the mutants that formed chromophore showed the same behaviour as wt rhodopsin, except for the mutant in helix I, Met44Thr, which showed a slightly altered behaviour. Although the spectrum after illumination is the same as that for the wt protein the spectrum after acidification showed an asymmetrical 440-nm band with a shoulder at about 400 nm. This is indicating that this band has two components, one main component at 440 nm but another minor component at lower wavelength presumably at 380 nm. This can be explained assuming that metarhodopsin II conformation obtained upon photobleaching has partly decayed, indicating that the stability of the active conformation of this mutant may be reduced as a result of the mutation.

Hydroxylamine reactivity and thermal stability

The reactivity of the wt and the mutants towards hydroxylamine in the dark was not significantly altered in the case of the different mutant proteins analysed as

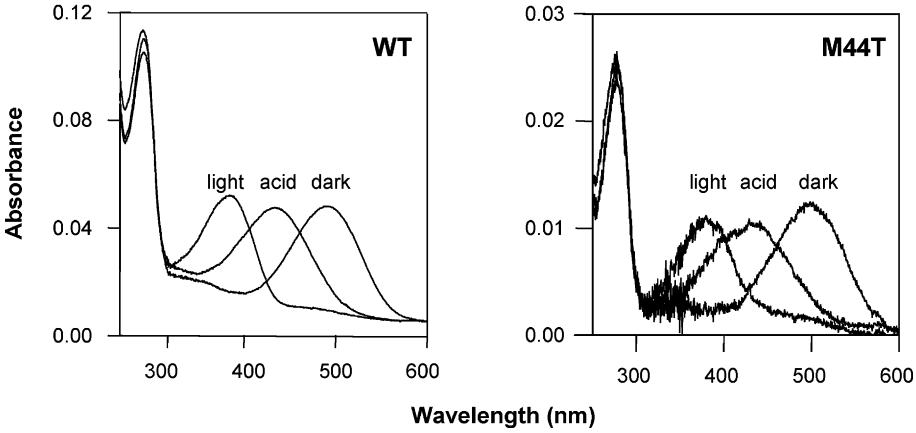

Fig. 4. Photobleaching and acidification behaviour of wt and the Met44Thr mutant. In each case the dark samples were illuminated with a $\lambda > 495$ nm light for 10 s and the corresponding spectrum was recorded. The samples were subsequently acidified after finishing the light spectrum and the acid spectra were immediately recorded.

indicated by the rate constants (k) summarised in Table 1. In the case of the thermal stability of the proteins at 55 °C, some differences could be observed among the different mutants (Table 1). The $t_{1/2}$ for the thermal decay process in the dark was calculated from the data obtained which were fitted to single exponential functions. These values are very similar for the wt and the Met44Trh mutant (12.0 and 11.7 min respectively), slightly decreased for the Val137Met and Leu328Pro mutants, and significantly decreased (about threefold) for the Arg135Leu mutant (Table 1).

Transducin activation

The functionally RP mutant proteins were measured by their ability to activate the G-protein transducin by means of a fluorescence assay [33]. The results were referred to the initial activation rate derived from the data points, corresponding to the first 60 s of activation. The results were normalised to the value of the wt taken as 1.00. Only the mutants that formed chromophore were assayed. Except for Arg135Leu, which showed reduced initial velocity of transducin activation, the other three mutants showed increased initial rates but to different extents. Leu328Pro showed similar activation rate to that for the wt, Val137Met showed about 25% increase, and Met44Thr showed an important increase of about 60% with regard to the value for wt (Table 1). No activity was detected when the mutants were assayed in the dark (data not shown).

Discussion

Mutants Gly114Asp in helix I, Pro171Gln in helix IV, His211Pro in helix V, and Gly106Trp in the first extracellular loop failed to regenerate with 11-*cis*-retinal. Gly114Asp had not been previously characterised but it had been proposed that it could not regenerate with retinal from the study of a series of mutants at position 114 [37]. In the case of Pro171Gln the lack of regeneration is consistent with the phenotype observed for the Pro171Leu mutant [25]. The other two mutants were previously characterised with similar results to the ones we obtain now, His211Pro was obtained in membranes [25] and in purified form [26] and Gly106Trp was classified as Type II mutant in a previous study [26]. The phenotype of all these mutants was very similar being characterised by very low expression of the proteins when compared to that of the wt and an anomalous electrophoretic behaviour. The presence of these lower bands, like the one for the Gly106Trp at about 28 kDa below the opsin band, has been associated with non-glycosylated species [38], and must be reflecting severe misfolding [27,28].

Four of the studied mutants regenerated with 11-*cis*-retinal to different levels when compared to the wt. Met44Thr in helix I regenerated essentially like the wt with an A_{280}/A_{500} ratio of 1.9 and the absorption visible maximum was slightly blue-shifted to 497 nm. Val137-Met, a mutation affecting a residue located close to the cytoplasmic boundary of helix III and Leu328Pro, affecting a residue in the cystoplasmic C-terminal tail of the protein, had slightly higher A_{280}/A_{500} ratios of 2.0 and 2.1, respectively. The λ_{max} of the chromophoric band did not suffer any shift and was located at 500 nm like that of the wt. In the case of the other mutant located in helix III Arg135Leu, in the vicinity of Val137-Met, the ratio was significantly increased to 2.5, indicating some degree of misfolding for this mutant but no change in the λ_{max} could be detected. This is in agreement with the previously reported spectrum of this mutant and different from other mutations at Arg-135, like Arg-135-Gly or Arg-135-Trp, that regenerated more and less than Arg135Leu, respectively [24]. This differential effect suggests that the size of the side chain at

position 135 may play a role in the folding of the protein. The spectral features of these four mutants were similar to those of wt rhodopsin except for the slight wavelength shift for the Met44Thr mutant and the increased ratio for the Arg135Leu mutant. These results indicate that the mutations did not directly affect the retinal binding pocket and that the electronic configuration around the retinal was not altered for the substitution except in the case of Met44Thr.

The thermal stability and the hydroxylamine reactivity in the dark were only significantly altered for the Arg135Leu mutant. This mutant showed an important decrease in thermal stability, with regard to the wt, of about threefold as indicated by their corresponding $t_{1/2}$ values (Table 1), and an increase in hydroxylamine reactivity in the dark. Arg-135 is part of a conserved triplet E(D)RY highly conserved in the GPCR superfamily. In particular, the Glu-134/Arg-135 pair is known to be very important in the activation of transducin [23,24]. Protonation of Glu-134 upon rhodopsin photoactivation is also thought to be one of the main features of the active conformation of the receptor [20]. This region is also in close contact with the region at the cytoplasmic boundary of helix VI in the vicinity of Glu-247 [13]. Arg-135 would be forming part of an ionic interaction with Glu-134 and Glu-247 at the top of transmembrane helices III and VI holding these two helices together at their cytoplasmic ends and maintaining the receptor in its inactive state as it has been recently shown for the β2-adrenergic receptor [39]. In our case, the Arg135Leu mutation would be disrupting this ionic interaction and this would result in partial misfolding and reduced ability of the mutant protein to bind 11-*cis*-retinal.

Mutants in the C-terminal tail, Leu328Pro and Ala346Pro, showed a similar phenotype to that of the wt with regard to the spectroscopic and functional properties analysed. In these cases the molecular defect underlying RP may be related to altered trafficking of the mutant proteins, resulting in mislocalisation of the mutant rhodopsins [40,41]. Mutations in the C-terminal tail of rhodopsin have been found to cause a faster progression of the disease in RP patients [42].

Mutants Met44Thr and Val137Met that regenerated chromophore showed increased transducin initial activation rate when compared to the wt. Only the Arg-135Leu mutant showed reduced transducin activation, this result being different from the one reported in a previous study where no activity could be detected [24]. This discrepancy could be due to the different conditions of the activation assay (rhodopsin concentration) in the two cases. Met44Thr showed a clear increase of approximately 60% in the initial activation rate. This is a mutation affecting a residue located in the central region of helix I in the transmembrane domain of rhodopsin. Met-44 is found close to the δ-carbon of the Lys-296 side chain and to the side chain of Thr-94 [13–15]. Thr-94 is a residue that has been found mutated to Ile in a case of the retinal disease Congenital Night Blindness [43]. The Thr94Ile mutant shows important spectroscopic and functional alterations [44,45] and it has been proposed to be involved in an electrostatic interaction with Glu-113 in the region of the protonated Shiff base linkage. In the case of Met44Thr, the mutation introduces a hydrophilic polar side chain possibly allowing electrostatic interaction with Thr-94 and indirectly affecting Glu-113 and the p*K*a of the Schiff base nitrogen facilitating its deprotonation [46].

In the case of the Val137Met mutant the increased activity may have a different explanation. From the crystal structure it is known that the three valines Val-137, Val-138, and Val-139 are forming a hydrophobic patch covering the ERY important motif. Arg-135 is important for transducin activation [23,24] and may be possibly involved in direct interaction with the α-subunit of transducin [47]. The observed results could be explained assuming a local conformational rearrangement, as a result of the Val137Met mutation, which allows the ER pair to be exposed thus facilitating transducin activation.

Thus, the two RP mutations Met44Thr and Val137-Met do not cause protein misfolding as it has been proposed as the molecular cause of retinitis pigmentosa for many mutations in the transmembrane domain of rhodopsin [33,34,36] and can be classified as Type I mutations, like the Phe45Leu mutation [26]. In the case of some of these Type I mutations, abnormal functioning resulting in faster activation kinetics could also play a role in retinitis pigmentosa by altering the optimal stoichiometric balance of the different proteins involved in the phototransduction biochemical reactions. As reported by different research groups, excessive phototransduction signalling [48,49] or defective levels of some proteins in the phototransduction process [50] may activate different apoptotic pathways and induce retinal degenerations like RP.

Acknowledgments

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