Retinoids Assist the Cellular Folding of the Autosomal Dominant Retinitis Pigmentosa Opsin Mutant P23H*

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The clinically common mutant opsin P23H, associated with autosomal dominant retinitis pigmentosa, yields low levels of rhodopsin when retinal is added following induction of the protein in stably transfected HEK-293 cells. We previously showed that P23H rhodopsin levels could be increased by providing a 7-membered ring, locked analog of 11-cis-retinal during expression of P23H opsin in vivo. Here we demonstrate that the mutant opsin is effectively rescued by 9- or 11-cis-retinal, the native chromophore. When retinal was added during expression, P23H rhodopsin levels were 5-fold (9-cis) and 6-fold (11-cis) higher than when retinal was added after opsin was expressed and cells were harvested. Levels of P23H opsin were increased ~3.5-fold with both compounds, but wild-type protein levels were only slightly increased. Addition of retinal during induction promoted the Golgi-specific glycosylation of P23H opsin and transport of the protein to the cell surface. P23H rhodopsins containing 9- or 11-cis-retinal had blue-shifted absorption maxima and altered photo-bleaching properties compared with the corresponding wild-type proteins. Significantly, P23H rhodopsins were more thermally unstable than the wild-type proteins and more rapidly bleached by hydroxylamine in the dark. We suggest that P23H opsin is similarly unstable and that retinal binds and stabilizes the protein early in its biogenesis to promote its cellular folding and trafficking. The implications of this study for treating retinitis pigmentosa and other protein conformational disorders are discussed.

Rhodopsin, the major pigment of vertebrate rod cells, is an integral membrane glycoprotein (~40 kDa) that functions as a G protein-coupled receptor in detecting dim light (1). Rhodopsin biogenesis involves the membrane insertion of opsin, a 348-amino acid polypeptide that must fold to form seven transmembrane α-helices and assemble with the chromophore, 11-cis-retinal. The chromophore is covalently attached to opsin by a protonated Schiff base linked to Lys296 in the seventh α-helix and interacts extensively with amino acid residues located in transmembrane α-helices (2). It also contacts residues at the base of the “retinal plug,” a short four-stranded β-sheet assembled from the N-terminal segment and the second extracytoplasmic loop of opsin (2). Given these intimate contacts, key questions are whether retinal can bind opsin at the early stages of its biogenesis and whether such binding increases the yield of the protein.

Retinal binding to opsin may be impaired in some forms of autosomal dominant retinitis pigmentosa (ADRP),1 a group of hereditary disorders that lead to rod photoreceptor death and subsequently to a severe loss of peripheral and night vision (3, 4). Approximately 150 mutations in opsin are implicated in this disease (5, 6). Based on heterologous expression studies (7–9), mutant opsins fall into three classes: Class I mutants bind retinal properly and accumulate at the cell surface; Class II mutants fail to bind retinal, are improperly glycosylated, and accumulate intracellularly; and Class III mutants bind retinal slightly, have less severe glycosylation defects than Class II mutants, and are found both on the cell surface and intracellularly. Class II and III mutants may have defects in the membrane insertion of opsin, opsin folding, retinal binding, or in the stability of the pigment once retinal has bound.

A potential strategy to treat ADRP caused by Class II and III rhodopsin mutants is to use retinal as a pharmacological chaperone (10), a ligand that corrects the misfolding of a protein by binding specifically to non-native or native forms. Pharmacological chaperones appear to act mainly in the endoplasmic reticulum (ER), releasing the proteins they target from the ER quality control machinery and increasing their flux to other cellular compartments (10). Because opsin stability in vitro is increased by the binding of 11-cis-retinal and other retinoids (11–13), these compounds may act as pharmacological chaperones and increase the yield of Class II and III rhodopsin mutants. In support of this idea, the level of T17M rhodopsin (a Class III mutant (8)) regenerated from cell membranes increased 10-fold when the protein was expressed in the presence of 11-cis-retinal (14). Cell surface expression of the Class III opsin, P23H, was increased in COS-7 cells when 9-cis-retinal was provided (15). Similarly, the addition of a 7-membered ring, locked, non-photoisomerizable form of 11-cis-retinal to P23H opsin during its expression promoted trafficking of the mutant protein and increased the yield of the pigment relative to the wild type.

1 The abbreviations used are: ADRP, autosomal dominant retinitis pigmentosa; ER, endoplasmic reticulum; WT, wild type; PBS, phosphate-buffered saline; DM, dodecyl-β-maltoside; λmax, visible absorption maxima; HPLC, high performance liquid chromatography; PNGase F, peptide N-glycosidase F; ConA, concanavalin A; TRITC, tetramethylrhodamine isothiocyanate; DAPI, 4’-6-diamidino-2-phenylindole hydrochloride; PCD, protein conformational disease.
to samples in which retinal was added after cell harvesting (16). Although these studies suggest that they can act as pharmacological chaperones for mutant opsins, further experiments are needed to establish the precise function of retinoids.

In this study, we show that the stimulation of P23H pigment production by added retinoids is not limited to the locked retinal used in our earlier work (16). We demonstrate that yields of rhodopsin can also be increased by 9- or 11-cis-retinal, the native chromophore. As observed with the locked retinal, the mutant pigment was formed efficiently only when retinoids were added during opsin expression. We show that 9- and 11-cis-retinal promote trafficking of P23H opsin through the secretory pathway to the cell membrane, indicating that retinoids bind the protein at an early stage. Finally, we show for the first time that P23H rhodopsin has a decreased thermal stability relative to wild-type (WT) rhodopsin, providing a partial explanation for the defect in its production.

EXPERIMENTAL PROCEDURES

Cell Culture, Regeneration, and Purification of Rhodopsin—Cell lines and growth conditions were described previously (16). All steps were carried out under dim red light. Retinals in 100% ethanol were added to a final concentration of 50 μM 2 h after growing cultures were induced with tetracycline (0.5 μg/ml) or after cells were harvested 48 h after induction. Cells were harvested and lysed in PBS (10 mM sodium phosphate buffer, pH 7.4, containing 1% n-dodecyl-β-maltoside (DM) (Anastrace), and rhodopsin was purified by immunofinity chromatography as described previously (16). Briefly, rhodopsin in cell lysates was bound to monoclonal antibody ID4, which recognizes the rhodopsin C terminus, and was coupled to Sepharose 4B (Amer sham Biosciences). The protein was eluted with a synthetic peptide corresponding to the last 18 amino acid residues of the rhodopsin C terminus in 10 mM sodium phosphate buffer, pH 6.0, or PBS, both containing 0.1% DM. UV-visible spectra of eluted pigments were recorded on a Tidas II spectrophotometer (World Precision Instruments). Visible absorption maxima (λ_max) were determined from the peak value of smoothed spectra. The variation in λ_max of two to four independently purified samples was less than ±1 nm.

Retinoid Analysis—All procedures were carried out under dim red light (>600 nm). Retinoids were analyzed following slight modifications of the procedures of Groenendijk et al. (17) and Smith and Goldsmith (18). Freshly purified rhodopsin was dried under a stream of nitrogen at room temperature. A 1:1 mixture of methanol and 1M hydroxylamine, pH 8.0. In an attempt to improve digestion, the following variations of this buffer were explored: a Nonidet P-40 concentration of 2.5 or 5% v/v; a 2-fold increase in both SDS and Nonidet P-40 concentrations; digestion of the reaction with a second aliquot of enzyme in an equal volume of buffer; incubation at 37°C with an identical buffer measuring pH 7.5 at room temperature; addition of 50 mM β-mercaptoethanol or dithiothreitol; a preincubation period of up to 5 h in a buffer lacking Nonidet P-40 followed by adjustment of Nonidet P-40 to 1% v/v and addition of enzyme; and the manufacturer’s recommended buffers. Untreated samples were identical except that water was added instead of PNGase F. In an attempt to improve digestion, we added a 10% SDS-polyacrylamide gel electrophoresis (PAGE) was performed, and the proteins were eluted with a synthetic peptide bound to monoclonal antibody 1D4, which recognizes the rhodopsin C terminus, and was coupled to Sepharose 4B (Amer sham Biosciences). The protein was eluted with a synthetic peptide corresponding to the last 18 amino acid residues of the rhodopsin C terminus in 10 mM sodium phosphate buffer, pH 6.0, or PBS, both containing 0.1% DM. UV-visible spectra of eluted pigments were recorded on a Tidas II spectrophotometer (World Precision Instruments). Visible absorption maxima (λ_max) were determined from the peak value of smoothed spectra. The variation in λ_max of two to four independently purified samples was less than ±1 nm.

Photobleaching—Photobleaching of recombinant rhodopsin samples was performed on a 150-watt fiber-optic light source (Dolan-Jenner) with a 495 nm filter. Photobleaching was performed in 0.1% DM in PBS, pH 7.4, and finally for 1 h with IRDye800-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) and DAPI (Molecular Probes). Cells were rinsed in PBST, mounted in 50 μl of anti-fade reagent (Molecular Probes) to retard photobleaching, and analyzed with a DMIRE2 Leica microscope.

Characterization of Rhodopsins—Photobleaching, hydroxylamine sensitivity, and thermal stability studies were conducted using a Cary 50 UV-visible spectrophotometer (Varian) and analyzed using Igor Pro software (WaveMetrics). Spectra were corrected for slight base-line displacements by subtracting the average absorbance from 625–650 nm. Photobleaching of P23H rhodopsin does not occur after 10 min incubation, and complete recovery is observed from the entire spectrum. Photobleaching was performed in 0.1% DM in 10 mM sodium phosphate, pH 6.0, at room temperature using a 150-watt fiber-optic light source (Dolan-Jenner) with a 495 nm filter. Single samples were illuminated for three 10-s intervals, 30 s, and 1 min, with scanning after each period. Hydroxylamine sensitivity was determined as described previously (16), except that 1 μl hydroxylamine, pH 6.0, was added to purified rhodopsin samples to a final concentration of 40 mM hydroxylamine. Spectra were multiplied by 1.04 to correct for the dilution because of hydroxylamine addition. Thermal stability was measured by obtaining full spectra every 2 min at 37°C in 0.1% DM in PBS, pH 7.4. The absorbance value at the absorption maximum was determined, corrected for base-line displacements, and normalized to the absorbance at the initial time point. Values from two independent experiments were averaged and fitted using Igor Pro with a monoexponential function for P23H samples and a linear function for WT samples.

RESULTS

Rationale—We showed previously that 11-cis-7-ring but not 11-cis-6-ring or 11-cis-9-demethyl-7-ring retinal increased the yield of P23H rhodopsin if administered during opsin synthesis (16). To test whether simpler retinoids, particularly the native chromophore, are capable of increasing the yield of P23H rhodopsin, we investigated the effects of 9- and 11-cis-retinal. The cellular expression, pigment characteristics, and cellular localization are reported here; full functional characterization of the proteins will be reported elsewhere.

Yield and Pigment Characteristics of WT Rhodopsin—Control experiments were conducted first with WT opsin to compare the yield of rhodopsin when retinals were added during opsin expression with the yield of rhodopsin when retinals were added after opsin had been expressed and cells harvested (post-harvest). Forty-eight hours after tetracycline induction, HEK-293 cells expressing WT opsin were harvested and incubated with both 9- and 11-cis-retinals. The rhodopsin formed was purified by immunofinity chromatography under conditions that selectively release folded opsin (19), and the first eluted

Rescue of P23H Opsin with 9- and 11-cis-Retinal

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fraction was examined by UV-visible spectroscopy and HPLC. The pigment obtained in post-harvest regeneration of WT opsin with 9-cis-retinal has a shifted absorption spectrum with $\lambda_{\text{max}} = 487$ nm (Fig. 1A, dashed line), whereas the pigment obtained with 11-cis-retinal has the characteristic $\lambda_{\text{max}} = 500$ nm of native rhodopsin (Fig. 1B, dashed line).

When retinals were added under dim red light to the medium of cells actively synthesizing WT opsin, the same spectral characteristics of the purified proteins were observed as in the post-harvest regeneration (Fig. 1, A and B, solid lines). The addition of 9-cis-retinal to the growth medium produced a pigment with $\lambda_{\text{max}} = 488$ nm, whereas 11-cis-retinal produced a pigment with $\lambda_{\text{max}} = 500$ nm. On acid denaturation (Fig. 1, A and B, insets) both proteins yielded a pigment with a $\lambda_{\text{max}}$ near 440 nm, indicating that retinal is linked as a Schiff base in the native protein (20). These pigments contained 9- and 11-cis-retinals.

Imunoaffinity purification indicated that the cellular yield of the WT protein was similar whether retinal was added during expression or post-harvest but was $\sim 30\%$ higher with 9- than with 11-cis-retinal (compare Fig. 1, A and B). To test whether these observations were representative of the total opsin in the cell, the opsin content of detergent-solubilized cell lysates was determined by quantitative immunoblotting. WT opsin yields increased only slightly (1.3- and 1.2-fold for 9- and 11-cis-retinal, respectively) when retinal was added during expression (Table I), results similar to the immunoaffinity purification. The level of WT opsin obtained in experiments with 9-cis-retinal was slightly less than that obtained with 11-cis-retinal ($\sim 0.8$-fold) and independent of whether the cofactor was added during expression or post-harvest. Thus, the effects of 9- and 11-cis-retinal on WT opsin and rhodopsin levels are relatively small.

**Yield and Pigment Characteristics of P23H Rhodopsin**—Parallel experiments were conducted with cells expressing P23H opsin. Cells were first harvested 48 h after induction with tetracycline and regenerated post-harvest with 9- or 11-cis-retinal, and the pigments were purified by immunoaffinity chromatography. The amount of pigment obtained with 9- or 11-cis-retinal (Fig. 1, C and D, dashed lines) was less than 10% of that obtained post-harvest with the WT protein.

Strikingly, when the individual retinals were added to cells during expression of P23H opsin, a significant amount of pigment was produced (Fig. 1, C and D, solid lines). The increase in P23H rhodopsin yield was similar for both retinals, reaching levels that were 5-fold (9-cis) and 6-fold (11-cis) higher than the
11-cis-retinal is the major chromophore bound to P23H rhodopsin (Fig. 1, insets). As in the case of the WT pigments, 11-cis-retinal is the major chromophore bound to P23H rhodopsin when 11-cis-retinal is added to the growth medium, whereas 9-cis-retinal predominates in pigments purified from cells grown in the presence of 9-cis-retinal (Fig. 1, C and D).

Analysis of the total yields of P23H opsin confirmed the results obtained by immunoaffinity purification. When 9- and 11-cis-retinals were added during expression, the P23H opsin level was increased by 3.5- and 3.4-fold, respectively, compared with the level when retinals were added post-harvest (Table I). Consistent with the increased yield of immunoaffinity purified P23H rhodopsin, P23H opsin levels were ∼50–70% higher with 9-cis- than with 11-cis-retinal (Table I).

Glycosylation State of Rhodopsin—To determine whether added retinals act early in rhodopsin biogenesis, we examined the glycosylation state of the purified protein. P23H rhodopsin purified from cells and exposed to 9- or 11-cis-retinal during expression acquires a high molecular mass smear above the major band at 36 kDa (Fig. 2A, lanes 2 and 4), similar to the WT protein (Fig. 2, A and B, lanes 1 and 3). The 36-kDa form (Fig. 2C, band A) and the high molecular mass smear in all samples correspond to glycosylated protein because they are converted by PNGase F to 33 kDa (Fig. 2C, band B) and 30 kDa species (Fig. 2C, band C). Band C (Fig. 2C) is not detected by ConA blotting (Fig. 2D) and therefore corresponds to deglycosylated opsin; band B (Fig. 2C) is more abundant in P23H samples. It is unlikely to be the result of incomplete PNGase F digestion because incubation for 72 h, a second addition of enzyme, or other changes in incubation conditions (see under “Experimental Procedures”) did not significantly alter the digestion pattern. Band B is glycosylated (Fig. 2D), but the glycan is apparently inaccessible or modified and is resistant to PNGase F. Although complete conversion to a deglycosylated form is not observed with PNGase F, the smear is clearly absent. The high molecular mass smear results from glycan modification by the Golgi apparatus (8). These results indicate that when P23H expression is induced in the presence of retinoids, an increased amount of the protein reaches the Golgi and is processed similarly to the WT protein. Thus, when they are added, 9- and 11-cis-retinals must interact with P23H opsin early during its biogenesis in either the ER or the Golgi apparatus.

In P23H rhodopsin samples a distinct 28-kDa immunoreactive band is observed (Fig. 2C, band D). This species is not shifted by PNGase F, suggesting that it does not contain an N-linked carbohydrate. Because this species is recognized by the ID4 antibody, it has an apparent molecular mass lower than that of deglycosylated opsin, and is not detected by N-terminal-specific antibody R2–15 (data not shown), it appears to be a degradation product formed by proteolysis near the N terminus.

Localization of Opin—To further test whether added retinals act at an early stage of rhodopsin biogenesis, we examined the distribution of WT and P23H opsin in cells exposed to 9- or 11-cis-retinal during expression of the protein. Immunofluorescence microscopy with ID4 antibody was used to detect opsin. Control experiments with uninduced WT and P23H cells lack an opsin-specific signal; only the DAPI staining of nuclei is detected (Fig. 3, A and E, blue fluorescence). The WT protein reaches the cell surface in the absence of added retinal (Fig. 3B) or in the presence of either 9- or 11-cis-retinal (Fig. 3, C and D) as observed by the presence of yellow-orange fluorescence on the cell periphery. In contrast, only a little P23H opsin is found at the cell surface in the absence of retinal, and distinct intracellular inclusions are observed (Fig. 3F). The inclusions appear to be aggresomes, similar to those observed previously in COS-7 and HEK-293 cells (15, 21). Strikingly, there are fewer inclusions and a substantial increase of P23H opsin at the cell surface with 9- or 11-cis-retinal (Fig. 3, G and H). Thus, adding either of the retinals to cells during opsin expression appears to promote the trafficking of the protein to the cell surface.

Characterization of Purified Rhodopsins—The increased yield of P23H rhodopsin in our experiments provides an opportunity to assess the physical and functional properties of the protein in detail. We therefore examined the photosensitivity, hydroxylamine sensitivity, and thermal bleaching of rhodopsin purified from cells to which retinals were added during opsin expression. Illumination with >495 nm light resulted in decreased absorbance in the visible region, indicating that all of the rhodopsin pigments, phototransformed (Fig. 4, A–D). A 10-s illumination converted the 9- and 11-cis WT rhodopsin to 380-nm species (Fig. 4, A and B), consistent with the formation of metarhodopsin II (22). Further illumination produced minor changes in the spectra of these proteins. In sharp contrast, a 10-s illumination of 9- and 11-cis P23H rhodopsin resulted in broad spectra (Fig. 4, C and D). Continued illumination of these pigments decreased absorbance near 480 nm with a slight increase at 380 nm. A similar but slower conversion was observed when the pigments were illuminated for 10 s and monitored in the dark (data not shown). The broad spectra may include species similar to the WT photointermediates metarhodopsin I (Meta I, , ) and II (Meta II, ). These results indicate that P23H rhodopsins have altered photo-bleaching properties.

Incubation of the detergent-purified samples with hydroxylamine, pH 6.0, revealed that 9- and 11-cis-retinal WT proteins were only slightly sensitive to this treatment (Fig. 4E, data for 9-cis not shown). However, detergent-purified 9- and 11-cis P23H pigments were both vulnerable to hydroxylamine attack.

![Table I](http://www.jbc.org/)
Rescue of P23H Opsin with 9- and 11-cis-Retinal

Fig. 3. Localization of opsin in WT and P23H cells. Permeabilized cells were probed with DAPI to detect nuclei (blue fluorescence) and 1D4 antibody to detect opsin (yellow-orange fluorescence). WT opsin was localized in the absence of retinal (B) or in the presence of 9- (C) or 11- (D) cis-retinal. Corresponding localization of P23H opsin is shown in F–H. A and E, uninduced WT and P23H cells.

Fig. 4. Photosensitivity and hydroxylamine reactivity of purified rhodopsins. WT and P23H pigments were prepared from cells to which retinals were added during opsin expression. A–D, photo-bleaching characteristics of purified WT (A and B) and P23H (C and D) rhodopsin containing 9- (A and C) or 11- (B and D) cis-retinal. Samples were illuminated with >485 nm light for a total of 10, 30, 120, or 0 s (WT, from top to bottom at 380 nm) or 0, 10, 30, and 120 s (P23H, from top to bottom at 500 nm). E–G, hydroxylamine reactivity of purified WT rhodopsin containing 11- (E) and P23H containing 9- (F) or 11- (G) cis-retinal. Untreated rhodopsin (solid lines) and rhodopsin after 4-h treatment with 40 mM hydroxylamine (dotted lines) are shown. H, hydroxylamine reactivity of P23H rhodopsin in cells. P23H rhodopsin was purified from cells expressing opsin in the presence of 9- or 11-cis-retinal without further treatment (bold and light lines, respectively) or treated with 0.5 M hydroxylamine for 1 h (dashed and dotted lines, respectively).

Fig. 5. Thermal stability of WT and P23H rhodopsin. Purified WT rhodopsin containing 9- (triangles) or 11- (inverted triangles) cis-retinal or P23H rhodopsin containing 9- (squares) or 11- (circles) cis-retinal was incubated at 37 °C in 0.1% DM in PBS, pH 7.4. The normalized absorbance value at the absorption maximum is plotted as a function of incubation time. The P23H data are fitted with a monoeXponential function; WT data are fitted with a linear function.

DiscusSion

We have demonstrated that 9- and 11-cis-retinals act as pharmacological chaperones to increase the yield of correctly folded P23H rhodopsin in HEK-293 cells. When these retinals were provided to cells post-harvest, the yield of pigment was very low, in agreement with earlier results (8, 16, 25). In contrast, when the retinals were added during opsin expression, P23H rhodopsin levels were increased 5–6-fold. Opioid levels were increased by ~3.5-fold under these conditions; the lower relative increase shows that a greater fraction of the protein folds correctly when retinal is added during expression.
The higher yield of P23H rhodopsin was the result of more efficient trafficking through the secretory pathway as evidenced by the increase in high molecular weight glycosylation of the purified protein and by the localization of P23H protein at the cell surface. The purified 9- and 11-cis P23H rhodopsins have a major spectral peak in the visible region and contain the expected retinal as the major chromophore. Thus, 9- and 11-cis-retinal are specific ligands that act early during biogenesis to increase the cellular yield of P23H opsin and rhodopsin, consistent with a role of these compounds as pharmacological chaperones.

Our results indicate that P23H rhodopsin has a structural alteration that decreases the stability of the protein. In contrast to earlier spectral studies (26), we observed an 8-nm shift in the visible absorption maximum with both 9- and 11-cis-retinal, suggesting that the structure of the protein near retinal is different from that in WT rhodopsin. The unusual response of the mutant proteins to light provides further evidence for an altered retinal environment. Most strikingly, 9- and 11-cis P23H rhodopsins have decreased thermal stability and increased hydroxylamine sensitivity, as observed with the locked analog of 11-cis-retinal (16). Prc3 is located on the intradiscal face of rhodopsin in close proximity to residues that form the retinal plug, and its substitution with His may disrupt this structure and alter the retinal environment, resulting in thermal instability.

To explain retinal rescue, we propose that the thermal stability of P23H opsin is decreased, similarly to P23H rhodopsin. Retinals may bind to native P23H opsin thereby stabilizing it, or they may bind non-native folding intermediates, acting as a scaffold for subsequent folding, and increase the rate at which these intermediates are converted to the native form. Such binding would prevent the protein from being recognized by the ER quality control machinery as misfolded, allowing it to escape degradation and promoting its transport to the Golgi apparatus. Although purified P23H rhodopsin in detergent is highly unstable, the protein accumulates because it is relatively stable in cell membranes.

Because our experiments examine steady-state levels of opsin rather than newly synthesized protein, we are unable to rule out the possibility that 9- and 11-cis-retinal might also act at a stage of biogenesis other than the secretory pathway. For example, retinals may influence the turnover and degradation of opsin at the cell surface, which has not been examined in HEK-293 cells. In support of this idea, when retinals are added to cells post-harvest, the level of P23H opsin is ~50% higher with 9-cis-retinal compared with 11-cis-retinal. Under these conditions, no new synthesis of opsin is expected, suggesting that the increase is because of decreased turnover of previously synthesized protein. Thus, reduced turnover may partially account for the increase in P23H rhodopsin levels when retinals are added during expression. Retinals may also increase P23H opsin yields by increasing opsin gene transcription or opsin translation. However, WT opsin levels were not increased significantly under any of the conditions examined, arguing against this possibility.

Thermal instability may be a common property of Class II and III mutant opsins. Previous studies (7, 8, 15, 25, 27, 28) of these proteins led to the conclusion that the mutant opsins were misfolded based on low pigment yield, intracellular accumulation, altered glycosylation, or non-native disulfide bonds but did not provide direct evidence for defects in protein thermal stability or folding kinetics. However, several mutant rhodopsins associated with ADRP were recently shown to exhibit decreased thermal stability (16, 29, 30); our studies of P23H rhodopsin provide another example. If thermal instability is common among ADRP mutant opsins, it may be possible to correlate the degree of instability with progress of the disease and to develop therapeutic approaches using retinoids as pharmacological chaperones.

The rescue of heterologously expressed mutant opsin by retinals raises important questions about rhodopsin biogenesis in the mammalian rod cell. First, at what stage of biogenesis does retinal bind newly synthesized opsin? Opsin biosynthesis is initiated in the rER segment of the rod, which contains the ER and Golgi apparatus (31), but the protein ultimately resides in the outer segment discs of the rod. Early autoradiographic and biochemical analyses (32, 33) in mice suggested that retinal binds to opsin only in the outer segment. However, subsequent experiments revealed that rhodopsin is present in the rough ER of rod cells, indicating that retinal binds to opsin in the inner segment (34). Our observation that retinals bind early during rhodopsin biogenesis is consistent with this view but does not resolve the contradiction between the earlier findings. Second, can other ADRP opsin mutants be rescued by retinals or other pharmacological chaperones? This intriguing possibility was first raised by Berson and co-workers (14), who demonstrated rescue of mutant T17M in mice with oral doses of vitamin A palmitate. Our results indicate P23H opsin is an excellent candidate for in vivo rescue.

ADRP caused by P23H opsin may be classified as a protein conformational disease (PCD). PCDs are mostly neurodegenerative disorders in which the misfolding of a specific protein generates non-native intracellular or extracellular aggregates correlated with a progressive loss of cell function and cell death (35–37). Other misfolded proteins, which are associated with diseases that are not formally classified as PCDs, have been found to aggregate in perinuclear structures termed aggresomes (38–40). Aggregate formation may therefore be a common attribute of disease-causing misfolded proteins. Because P23H opsin is conformationally unstable and forms aggresomes (15, 21), it may be considered a cause of PCD. The results presented here along with our earlier work (16) thus support the idea that an effective therapeutic strategy for PCDs may be to correct the misfolding of the underlying protein (36, 41).

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