Cysteine Residues of Photoreceptor Peripherin/rds: Role in Subunit Assembly and Autosomal Dominant Retinitis Pigmentosa†

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ABSTRACT: Peripherin/rds is a tetraspanning membrane glycoprotein that is essential for the morphogenesis and stabilization of outer segments of vertebrate rod and cone photoreceptor cells. Mutations in the gene for peripherin/rds are responsible for retinal degeneration in the *rds* mouse and a variety of progressive human retinal degenerative diseases including autosomal dominant retinitis pigmentosa and macular dystrophy. Peripherin/rds associates with rom-1, a homologous subunit, to form a heterotetrameric complex. This study examines the importance of cysteine residues for the structure of peripherin/rds and its assembly with rom-1. Each of the 13 cysteine residues in bovine peripherin/rds was individually replaced with a serine residue by site-directed mutagenesis, and the resulting mutants were expressed individually or together with rom-1 in COS-1 cells. SDS−polyacrylamide gel electrophoresis, immunoprecipitation, and velocity sedimentation were carried out to evaluate the ability of these mutants to form disulfide-linked homodimers, associate with rom-1, and assemble into tetramers characteristic of wild-type peripherin/rds. Substitution of each of the six nonconserved cysteines had no apparent effect on dimer formation, folding, or subunit assembly. In contrast, replacement of any of the seven conserved cysteine residues predicted to lie within a 150 amino acid intradiscal loop significantly altered these properties. Six of these mutants, including a C214S mutant linked to autosomal dominant retinitis pigmentosa, were unable to fold normally, interact with rom-1, or self-assemble into tetramers but instead formed a mixture of large aggregates and a smaller component, most likely a dimer. The C150S mutant, on the other hand, was incapable of forming intermolecular disulfide bonds but did associate with rom-1 into a heterotetramer. These results suggest that (1) the conserved C150 residue is required for intermolecular disulfide bonding but not subunit assembly; (2) the six other conserved cysteine residues are crucial for proper folding and subunit assembly, possibly through formation of intramolecular disulfide bonds; and (3) the misfolding and defective subunit assembly of the C214S mutant is responsible for a form of monogenic autosomal dominant retinitis pigmentosa.

Peripherin/rds is an integral membrane glycoprotein that forms an oligomeric complex with a nonglycosylated, homologous subunit, rom-1, at the rim region of rod and cone photoreceptor outer segment disc membranes (*1*). Primary structural analysis and immunocytochemical labeling studies indicate that peripherin/rds spans the lipid bilayer four times and has its amino and carboxyl termini exposed on the cytoplasmic side of the disc membrane (*2*). A large intradiscal loop of approximately 150 amino acids joins the third and fourth transmembrane segments and contains seven highly conserved cysteine residues and an N-linked glycosylation site (see Figure 1). Immunoprecipitation and velocity sedimentation analysis indicate that peripherin/rds associates with rom-1 to form a heterotetrameric complex (*3*, *4*). In one model, this complex is thought to consist of a disulfide-linked homodimer of peripherin/rds noncovalently

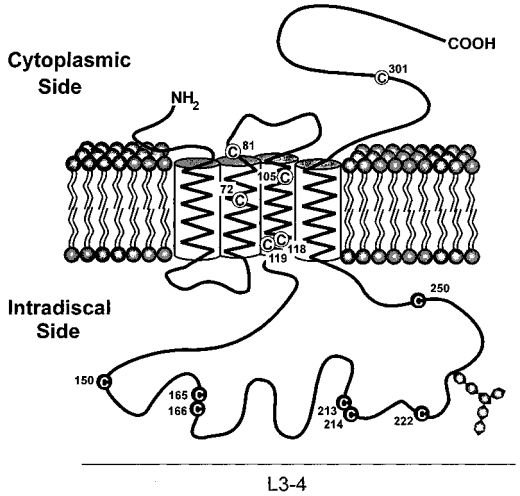

FIGURE 1: Current topological model for peripherin/rds in disc membranes (*1*). Nonconserved cysteines (open circles) are at positions 72, 81, 105, 118, 119, and 301; conserved cysteines (shaded circles) are present within the intradiscal L3−4 loop at positions 150, 165, 166, 213, 214, 222, and 250.

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bound to a disulfide-linked homodimer of rom-1 (*5*). Studies of Bascom et al. (*3*) also suggest that both peripherin/rds and rom-1 contain intramolecular disulfide bonds.

Peripherin/rds is essential for the formation and stabilization of normal photoreceptor outer segments since *rds* mice homozygous for the disrupted RDS gene (*rds/rds*) fail to form photoreceptor outer segments and heterozygous *rds* mice (*rds*/+) develop shortened, highly disorganized outer segments (*6*−*10*). Mutations in the gene for peripherin/rds have also been linked to a variety of progressive human retinal degenerations, including autosomal dominant retinitis pigmentosa (RP)[1] (*11*−*18*), digenic RP (*19*), retinitis punctata albescens (*20*), macular dystrophy (*16*, *18*, *21*), and fundus flavimaculatus (*18*). The majority of these mutations result in single amino acid substitutions or deletions in the large intradiscal loop of peripherin/rds. Several cases of autosomal RP have also been linked to cysteine mutations in peripherin/rds (*11*, *14*, *16*).

Recently, a heterologous COS-1 cell system has been described to study the cellular expression and molecular properties of peripherin/rds and rom-1 (*22*). This system has been used to evaluate the role of subunit interactions in a digenic form of autosomal dominant RP (*5*). On the basis of these studies, a subunit assembly model has been proposed that stresses the functional importance of peripherin/rds-containing tetramers on the formation and stabilization of photoreceptor outer segments. Reduced levels of such tetramers are suggested to result in unstable, disorganized outer segments and progression of a RP disease phenotype.

In this study, the COS-1 cell system has been used with site-directed mutagenesis to examine the importance of the various cysteine residues in the structure and subunit assembly of peripherin/rds. The results of this study provide new insight into the role of specific cysteine residues in disulfide-linked homodimerization, protein folding, and subunit interactions. The effect of a C214S mutation, linked to a monogenic form of autosomal dominant RP, on the molecular properties and subunit assembly of peripherin/rds is also reported.

MATERIALS AND METHODS

Site-Directed Mutagenesis. Plasmids composed of the pcDNAI/AMP vector (Invitrogen, San Diego, CA) and the full-length coding regions for bovine peripherin/rds (pcPER) or rom-1 (pcROM) were used for heterologous (COS-1) cell expression as previously described (*22*). A PCR-based method of primer-directed mutagenesis (*23*) was used to generate missense mutations in which individual cysteine residues were replaced with serine residues; subcloned portions of the WT pcPER were used as templates.

Plasmid pHindbg was constructed by ligating a 260 base-pair (bp) *Hind*III/*Bgl*II fragment of the peripherin/rds coding region (5′ region) into a *Hind*III/*Eco*RI-digested Bluescript II KS+ plasmid (Stratagene, La Jolla, CA) using a synthetic *Bgl*II/*Eco*RI adapter (5′-GATCTGGTCACCG-3′, 5′-AATTCGGTGACCA-3′). Plasmid pBgSks was constructed by ligating a 416 bp *Bgl*II/*Sac*II fragment of the peripherin/rds coding region (central part) directly into the Bluescript II KS+ plasmid polylinker. Plasmid pScXho was constructed by ligating a 521 bp *Sac*II/*Xho*I fragment of the peripherin/rds coding region (3′ region) directly into the Bluescript II SK+ plasmid polylinker. Sense-strand synthetic oligonucleotides (BRC Facility, Vancouver, BC) and the templates used for the corresponding mutagenesis reactions are shown in Table 1.

Table 1: Synthetic Oligonucleotides Used for PCR Mutagenesis

subclone	mutation	synthetic oligonucleotide
pHindBg	C72S	5′-CTGTCCTCTGTCTTCAATTCTCTGG-3′
pBgSks	C81S	5′-GGCAAGATCTCTTACGACGCCC-3′
	C105S	5′-GCCGTGTCTGTCCTCTTCAACG-3′
	C118S	5′-GGCCCTCAGCTGCTTCCTCCTGC-3′
	C119S	5′-GCCCTCTGCAGCTTCCTCCTGCG-3′
	C150S	5′-CCAGGCCGGTCTTTCATGAAG-3′
	C165S	5′-GATCGAATTCAAGAGCTGCGG-3′
	C166S	5′-CGAATTCAAGTGCAGCGGCAAC-3′
	C213S	5′-CCCTTCAGCTCCTGCAACCC-3′
	C214S	5′-TTCAGCTGCAGCAACCCCAAC-3′
pScXho	C222S	5′-CCGCGGCCCTCGATCCAGTACC-3′
	C250S	5′-CTGCGTGGCAGCAGGGCC-3′
	C301S	5′-GACCCTGAGTCCGAGAGTGAGG-3′

PCR products obtained essentially as described (*23*) were digested with the appropriate restriction enzymes, purified from agarose gels, and cloned into their respective template vectors (see above and Table 1). The desired mutations were selected by complete (single-strand) dideoxy-sequencing with a Sequenase T7 kit (USB). The mutagenized regions were subsequently subcloned back into the WT peripherin/rds gene and final expression constructs were confirmed by restriction mapping.

Heterologous Expression. COS-1 cells (ca. 2×10^5/ 60 mm dish) were singly transfected with expression plasmid (12 μg) or cotransfected with a mixture of two expression plasmids (6 μg each) and solubilized with 1% Triton X-100 in phosphate-buffered saline 72 h posttransfection, essentially as described (*22*). Extracts were subjected to SDS−polyacrylamide gel electrophoresis under reducing and nonreducing conditions (presence and absence of 2-mercaptoethanol), and peripherin/rds expression was detected on western blots.

Immunoprecipitation and Velocity Sedimentation. Homomeric peripherin/rds and heteromeric peripherin/rds−rom-1 complexes were isolated from COS-1 cell extracts by an immunoprecipitation procedure (*5*). Briefly, Triton X-100-solubilized cell extracts were incubated with anti-peripherin/rds Mab per2B6 coupled to Sepharose beads. The matrix was washed to remove unbound proteins, and the bound peripherin/rds complex was then eluted with an excess of 2B6 peptide corresponding to the C-terminal nine amino acids of bovine peripherin/rds.

For velocity sedimentation studies, the purified peripherin/rds complex was applied to a 5−20% (w/w) sucrose gradient in the presence of 1 mM DTT and centrifuged in a Beckman TLS-55 rotor for 16 h at 50 000 rpm. Sedimentation profiles of fractionated sucrose gradients were determined by western blotting and laser densitometry according to Goldberg and Molday (*5*).

Calculation of Sedimentation Coefficients. $S_{20,w}$ estimates were made as described originally (*22*) but with the following modifications: all partial specific volume values were

[1] Abbreviations: bp, base pair; Mab, monoclonal antibody; RP, retinitis pigmentosa; WT, wild-type.

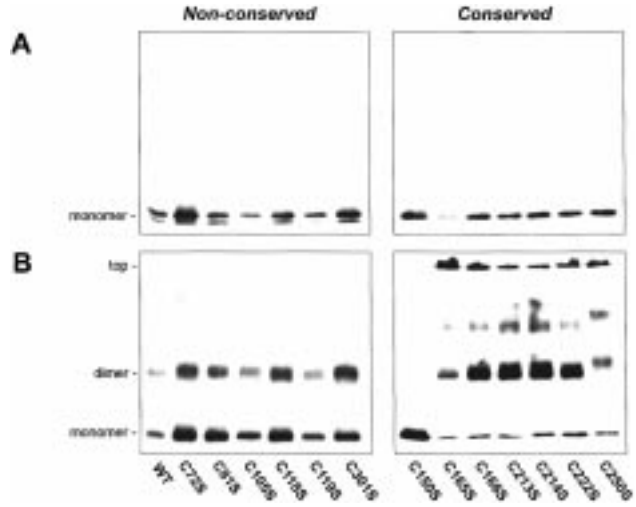

FIGURE 2: Expression and dimerization of WT and mutant peripherin/rds. COS-1 cells transfected with the indicated plasmids were detergent-solubilized 72 h posttransfection, and approximately 1.5 μg of total protein/lane was subjected to SDS−polyacrylamide gel electrophoresis in the presence (A) or absence (B) of reducing agent (2-mercaptoethanol). Western blots were labeled with anti-peripherin/rds Mab per2B6 for chemiluminescent detection.

assumed to be 0.83 mL/g [determined for the bovine peripherin/rds−rom-1 complex (*4*)], initial sample radius (r_{min}) was taken as 47.5 mm (Beckman TLS-55 rotor r_{min} corrected for sample and gradient volume), and gradient height was measured as 29 mm.

Western Blots. Expression, dimerization, and assembly of peripherin/rds and peripherin/rds−rom-1 complexes were detected on western blots labeled with either the anti-peripherin/rds Mab per2B6 or the anti-rom-1 Mab rom1C6 followed by goat anti-mouse Ig−peroxidase as previously described (*22*, *24*). The enhanced chemiluminescence (ECL) system (Amhersham, Canada) was used for detection of labeled proteins.

RESULTS

Location of Cysteine Residues in Peripherin/rds. Previous studies have demonstrated the utility of a heterologous expression system for mutagenesis-based structural studies of peripherin/rds (*5*, *22*). This system has now been used to examine the relative importance of the various cysteine residues found in bovine peripherin/rds.

Figure 1 depicts the current topological model for peripherin/rds (*1*, *2*) in the outer segment disc membrane and highlights the position and conservation of the 13 cysteine residues present in the bovine sequence. Residues shaded in black are absolutely conserved in both peripherin/rds and its homologous subunit, rom-1, across all species examined to date (*2*, *3*, *8*, *25*−*31*). These seven conserved cysteines are confined to the large intradiscal L3−4 loop joining the third and fourth transmembrane segments. Nonconserved residues are present in both the putative transmembrane segments and segments exposed on the cytoplasmic side of the membrane.

Expression and Dimerization of Peripherin/rds Cysteine Mutants. In order to assess the effect of the various mutations on the properties of peripherin/rds, each of the 13 cysteine residues in bovine peripherin/rds has been individually substituted with serine. As shown in Figure 2A, all of the variants are expressed in COS-1 cells and migrate as monomers (apparent M_r 35K) on SDS−polyacrylamide gels under reducing conditions. Although relative protein expression levels varied between experiments (a common property of transient transfection systems), none of the mutants showed consistently altered levels of expression relative to WT. These observations indicate that none of the cysteine residues is absolutely required for the biosynthesis of peripherin/rds in COS-1 cells.

The mobilities of the peripherin/rds mutants were also compared under nonreducing conditions to assess the ability of these cysteine variants to form disulfide-linked dimers. As shown in Figure 2B (left panel), a substantial portion of the WT and nonconserved cysteine mutants migrate at approximately twice the molecular weight of the monomeric form of peripherin/rds, as previously reported for peripherin/rds from rod photoreceptor outer segment membranes (*32*). The conserved cysteine mutants (Figure 2B, right panel), on the other hand, show distinctly different behaviors. Peripherin/rds with mutations at positions 165, 166, 213, 214, 222, and 250 form mainly dimers and aggregates found at the top of the gel. In contrast, the conserved C150S mutant migrates solely as a monomer under nonreducing, as well as reducing, conditions.

These results indicate that nonconserved cysteine mutants, like WT peripherin/rds, are capable of forming disulfide-linked dimers. Conserved C165S, C166S, C213S, C214S, C222S, and C250S mutants also form dimers under nonreducing conditions but in addition have a strong tendency to form larger aggregates, most likely due to improper folding of these mutants. The inability of the C150S to form disulfide-linked dimers in presence or absence of reducing agents suggests that this cysteine residue is essential for intermolecular disulfide bond formation. Three nonconserved cysteine mutants (C72S, C118S, and C119S), two conserved cysteine mutants (C165S and C214S), and the intermolecular disulfide dimerization-defective mutant (C150S) were selected for further study.

Interaction of Peripherin/rds Cysteine Mutants with Rom-1. Coimmunoprecipitation studies have previously established that peripherin/rds assembles with rom-1 in membranes of both vertebrate photoreceptors and transiently cotransfected COS-1 cells (*3*, *22*, *24*). This procedure has now been used to assess the ability of the nonconserved, conserved, and C150S peripherin/rds cysteine mutants to assemble with WT rom-1.

COS-1 cells were transiently cotransfected with peripherin/rds and rom-1 plasmids and the detergent-solubilized cell extracts were immunoprecipitated with a per2B6−Sepharose matrix. Western blots of the cell extracts and unbound and bound fractions were subsequently labeled with anti-peripherin/rds and anti-rom-1 antibodies to assess the interaction of WT and mutant peripherin/rds with rom-1. As shown in Figure 3, peripherin/rds immunoreactivity was observed in the bound fraction but not in the unbound fraction, indicating that the per2B6−Sepharose matrix quantitatively precipitated WT and the various cysteine peripherin/rds mutants from COS-1 cell extracts. Analogous western blots labeled with an anti-rom-1 antibody (Rom 1C6) further show that over 90% of the rom-1 coprecipitates with WT peripherin/rds and the nonconserved C118S mutant (Figure 3). Similar results were obtained for the nonconserved C72S and C119S

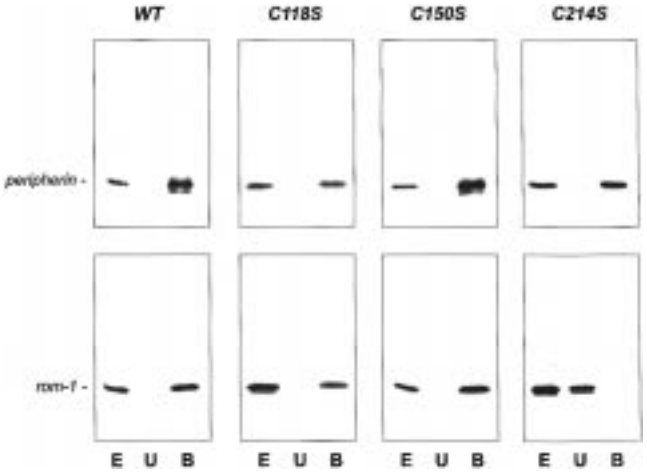

FIGURE 3: Coassembly of peripherin/rds cysteine mutants with rom-1. COS-1 cells were cotransfected with wild-type (WT) or mutant peripherin/rds plasmid and WT rom-1 plasmid. Detergent-solubilized extracts (E) were adsorbed to per2B6−Sepharose, and after removal of the unbound fractions (U), the bound fractions (B) were eluted with the 2B6 competing peptide. Samples were analyzed on western blots labeled with either the peripherin/rds Mab per2B6 (top panel) or the rom-1 Mab rom1C6 (bottom panel). Rom-1 coprecipitates with WT and the C118S and C150S mutants but not with the C214S mutant.

mutants (data not shown). Interestingly, rom-1 also coprecipitates with the C150S disulfide dimer-defective mutant. In contrast, rom-1 does not coprecipitate with the C214S (Figure 3) or C165S conserved mutants (data not shown) but instead is present only in the unbound fraction.

These studies indicate that the interaction of peripherin/rds with rom-1 requires cysteines at conserved positions 165 and 214 but not at nonconserved postions 72, 118, or 119. Replacement of cysteine with serine at the conserved position 150 prevents disulfide-linked homodimerization but does not prevent peripherin/rds from noncovalently associating with rom-1.

Velocity Sedimentation Analysis of the Peripherin/rds Cysteine Mutants. Previous studies have shown that the WT peripherin/rds−rom-1 complex from rod outer segment and COS-1 cell membranes sediments as a heterotetrameric complex (*4*, *22*). In the absence of rom-1, WT peripherin/rds self-assembles into a homotetrameric protein. This form of the protein is thought to compensate for reduced levels of the heterotetrameric protein in individuals who inherit a null allele of rom-1 (*5*).

We have examined the sedimentation behavior of several conserved and nonconserved peripherin/rds cysteine mutants expressed individually or with rom-1. The sedimentation profiles and sedimentation coefficients are given in Figure 4 and Table 2. The nonconserved cysteine mutants exhibit sedimentation profiles and sedimentation coefficients similar to WT peripherin/rds in both the presence and absence of rom-1. Interestingly, the disulfide-linked dimer-defective C150S mutant also has a sedimentation profile and coefficient similar to WT in the presence and absence of rom-1. In contrast, the conserved C214S and C165S mutants, which fail to assemble with rom-1, sediment more slowly than WT peripherin/rds when singly expressed in COS-1 (Figure 4 and Table 2). In addition, a significant, but variable, fraction of these mutants sediments near or at the bottom of the centrifuge tube, presumably due to aggregation.

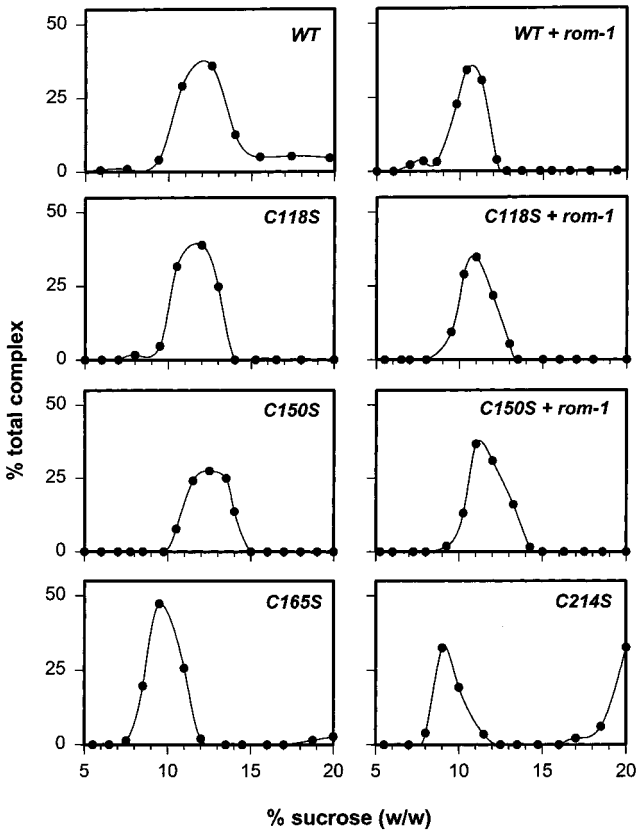

FIGURE 4: Velocity sedimentation analysis of peripherin/rds cysteine mutants. COS-1 cells were transfected with peripherin/rds wild-type (WT) or mutant plasmids alone (C118S, C150S, or C165S) or together with rom-1 (WT + rom-1, C118S + rom-1, or C150S + rom-1). Peripherin/rds-containing complexes were purified on a per2B6−Sepharose matrix and sedimented through a 5−20% sucrose gradient in the presence of 1 mM DTT. Fractions were assayed on western blots for peripherin/rds with per2B6 antibody for singly transfected cells and with rom1C6 antibody for cotransfected cells and quantified by laser densitometry. Velocity sedimentation studies of the C165S and C214S mutants coexpressed with rom-1 were not carried out since these mutants do not associate with rom-1. The WT profiles were from ref *5*.

These results indicate that replacement of the nonconserved cysteine residues with serine does not affect peripherin/rds subunit assembly. In contrast, replacement of the conserved cysteines (with the exception of cysteine at position 150) result in proteins that fail to assemble into tetramers, presumably due to improper folding. The C150S mutant is unique in that replacement of this conserved cysteine affects disulfide-linked dimerization but does not affect its assembly into homo- and heterotetramers.

DISCUSSION

In this study the contribution of the various cysteine residues to the folding and subunit assembly of peripherin/rds has been determined by individually replacing each cysteine with a serine residue. The 13 cysteine residues in bovine peripherin/rds fall into two groups according to their conservation and effect on protein structure. Group I consists of the six nonconserved cysteine residues present in the putative transmembrane and cytoplasmic segments of peripherin/rds (positions 72, 81, 105, 118, 119, and 301), while group II contains seven highly conserved cysteines found in the intradiscal loop (positions 150, 165, 166, 213, 214, 222, and 250).

Table 2: Sedimentation Coefficients for Peripherin/rds and Peripherin/rds-rom-1 Complexes[a]

variant	$S_{20,w}$ −rom-1	$S_{20,w}$ +rom-1	conservation/ location
WT[b]	5.36 ± 0.14 (n = 3)	4.99 ± 0.37 (n = 4)	
C72S	5.13 ± 0.41 (n = 3)	4.77 ± 0.09 (n = 3)	nc/TM2
C118S	5.38 ± 0.05 (n = 3)	5.06 ± 0.05 (n = 3)	nc/TM3
C119S	5.47 ± 0.18 (n = 3)	5.17 ± 0.07 (n = 3)	nc/TM3
C150S	5.18 ± 0.31 (n = 3)	4.99 ± 0.23 (n = 3)	c/L3−4
C165S	3.91 ± 0.14 (n = 3)	not assembled	c/L3−4
C214S	3.92 ± 0.19 (n = 3)	not assembled	c/L3−4

[a] Sedimentation coefficients estimated as described in the Materials and Methods are given for WT and mutant peripherin/rds and peripherin/rds−rom-1 complexes. Values for WT, C72S, C118S, C119S, and C150S mutants are similar; the WT peripherin/rds−rom-1 complex from rod outer segments has been shown to be a tetramer (*4*). Values for C165S and C214S expressed in the absence of rom-1 are considerably less than that for the tetrameric form of peripherin/rds. c, conserved; nc, nonconserved; TM2, transmembrane segment 2; TM3, transmembrane segment 3; L3−4, intradiskal loop joining transmembrane segments 3 and 4 (see Figure. 1). [b] The sedimentation behavior of WT peripherin/rds and peripherin/rds−rom-1 has been characterized previously (*22*). Sedimentation coefficients for these species have been recalculated from the original data using new values for (1) the partial specific volume of the protein−detergent complex and (2) sucrose gradient geometry, as described in the Materials and Methods. We consider that these adjustments more accurately reflect absolute $S_{20,w}$ values as measured.

Substitution of group I cysteines has no apparent effect on the formation of disulfide-linked homodimers as analyzed by SDS−polyacrylamide gel electrophoresis under nonreducing conditions. Immunoprecipitation studies and velocity sedimentation analyses of three of these mutants (C72S, C118S, and C119S) indicate that, like WT peripherin/rds, they assemble into heterotetramers or homotetramers in the presence or absence of rom-1, respectively. It is likely that the other three nonconserved cysteines behave similarly. On this basis, one can conclude that the nonconserved cysteine residues do not play an important role in the folding or subunit assembly of peripherin/rds and are not likely to be involved in disulfide bonding.

The seven conserved cysteine residues of group II appear to play an important role in the structure of peripherin/rds. Six of the seven group II cysteine residues (positions 165, 166, 213, 214, 222, and 250**)** are required for proper folding and subunit assembly of peripherin/rds. Individual replacement of these cysteine residues with serine results in mutant proteins that do not assemble into a nativelike tetramer. Both SDS−polyacrylamide gel electrophoresis and velocity sedimentation studies reveal that these mutants exist as a mixture of large protein aggregates and a distinct species, most likely a dimer. The intradiscal compartment in which these conserved cysteines reside is topologically similar to the extracellular space and is expected to maintain a nonreducing environment. Therefore, it is possible that some or all of these six cysteine residues participate in intramolecular disulfide bonds.

The presence of intramolecular disulfide bonds in peripherin/rds previously has been suggested from the studies of Bascom et al. (*3*). Intramolecular disulfide bonds in integral membrane polypeptides are known to be important for both proper folding and subunit assembly. For example, vertebrate rod and cone opsins have an intramolecular disulfide bond between the third transmembrane helix and second intradiscal loop that is essential for proper protein folding, transport, stability, and light-dependent activation of transducin (*33*, *34*). Assembly of the GLUT-1 glucose transporter into tetramers also depends strongly on the presence of intramolecular disulfide bonds located in the extracellular domains (*35*). In the case of peripherin/rds, the misfolding and abnormal subunit assembly observed for the six group II cysteine mutants may result from the disruption of intracellular disulfide bonds. Replacement of the cysteine residues with more polar serine residues, however, may also contribute to the abnormal structural properties observed in these peripherin/rds mutants.

The cysteine at position 150 is unique among the group II conserved cysteine residues in that the C150S mutant is incapable of forming disulfide-linked homodimers but does assemble into a tetramer in the presence or absence of rom-1 as determined by velocity sedimentation analysis. This single cysteine, therefore, is necessary and sufficient for disulfide-linked homodimerization. Interestingly, the folding and subunit association of peripherin/rds does not require this intermolecular disulfide bond; instead subunit assembly appears to be mediated primarily through noncovalent interactions. This somewhat surprising result is not unprecedented. Recent studies have shown that cleavage of the intersubunit disulfide bridges in bovine dopamine β-monooxygenase does not affect that enzyme's tetrameric subunit stoichiometry or activity (*36*). The same study also concludes that a lack of intersubunit disulfide bonds significantly destabilizes the tetrameric structure, a result that might be predicted from purely thermodynamic considerations. Structural stability of other multisubunit proteins has also been shown to be dependent upon the presence of intersubunit covalent bonds (*37*). These considerations suggest that the intermolecular disulfide bond at position 150 of peripherin/rds may contribute to the stability of the tetrameric protein complex. Alternatively, these disulfide bonds may not be directly involved in tetramer formation but instead may link the tetramers into higher order species that are not observed under the conditions used for the velocity sedimentation experiments reported here. The relative stability of WT peripherin/rds and various mutants and their possible ability to form larger oligomeric species are currently under investigation.

The C214S mutation in peripherin/rds has been linked to a monogenic form of autosomal dominant RP characterized by a progressive degeneration of photoreceptors (*14*). Studies presented here indicating that this mutant is misfolded and incapable of forming heterotetramers with rom-1 are consistent with its involvement in photoreceptor degeneration and support the subunit assembly model for autosomal dominant RP (*5*). In the simplest case the disease state can result from decreased levels of peripherin/rds-containing tetramers in individuals heterozygous for the C214S mutation. Reduced levels of peripherin/rds have been shown to result in shortened, highly disorganized outer segments and lead to slow retinal degeneration in heterozygous *rds* mice (*7*). Haploinsufficient levels of peripherin/rds-containing tetramers have been suggested to account, at least in part, for the digenic form of RP resulting from coinheritance of a L185P peripherin/rds gene and a null allele of rom-1 (*5*). Although reduced levels of peripherin/rds-containing tet-

ramers can lead to a disease phenotype, the presence of the misfolded C214S mutant may also contribute to the disease phenotype.

Missense mutations in the other conserved cysteine residues have not yet been reported. Their occurrence is predicted to cause autosomal dominant RP. In contrast, missense mutations in the nonconserved cysteines are expected to have little, if any, effect. Indeed, recently Fishman et al. (*38*) have reported that individuals with a missense mutation in the nonconserved cysteine of codon 72 (C72S mutation) are normal. These authors have ascribed this mutation as a polymorphism.

Autosomal dominant RP has also been linked to a deletion mutation at the nonconserved 118 or 119 position of peripherin/rds (*11*). Studies presented here showing that a serine substitution at either of these nonconserved positions has no obvious effect on peripherin/rds structure or assembly suggests that the deletion of an amino acid, and not the absence of a cysteine residue, is primarily responsible for the disease. Indeed, preliminary heterologous expression studies of the C118del (C119del) peripherin/rds variant indicate that this mutant is highly misfolded (C. Loewen and R. Molday, unpublished results). The shortening of the polypeptide chain, and not loss of cysteine functionality, appears to prevent the proper folding of this mutant.

In conclusion, the present mutagenesis study indicates that the conserved cysteine residues within the large intradiscal loop play an important role in the structure of peripherin/rds. Six of these cysteine residues are essential for proper protein folding and subunit assembly, possibly through their involvement in intramolecular disulfide bond formation. Abnormal folding and subunit assembly of the C214S mutant appear to be responsible for the monogenic form of autosomal dominant RP linked to this mutation. The conserved cysteine residue at position 150 is required for disulfide-dependent homodimerization but is not required for hetero- or homotetramer formation. Since rom-1 is highly homologous to peripherin/rds, we expect that conserved cysteine residues in the large intradiscal loop of rom-1 likewise play an essential role in the folding and subunit assembly of this subunit.

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