Subunit Composition of the Peripherin/rds−Rom-1 Disk Rim Complex from Rod Photoreceptors: Hydrodynamic Evidence for a Tetrameric Quaternary Structure[†]

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ABSTRACT: Peripherin/rds and rom-1 are homologous integral membrane protein subunits found as an oligomeric complex at the rim regions of rod and cone photoreceptor outer segment disks. These proteins are essential for the morphogenesis of normal outer segments and have been linked to a variety of human retinal degenerative diseases. Previous studies have suggested that disulfide-linked homodimers of peripherin/rds and rom-1 can associate noncovalently to form higher order structures. We have characterized the hydrodynamic properties of Triton X-100 solubilized peripherin/rds−rom-1 complexes from bovine ROS membranes by gel exclusion chromatography on Sepharose Cl-6B and velocity sedimentation through H_2O- and D_2O-based sucrose gradients. A single hydrodynamic species is observed which has a Stokes radius of 6.2 nm, a sedimentation coefficient ($S_{20,w}$) of 5.8 S, and a partial specific volume of 0.83 mL/g. From these data the molecular mass of the detergent-peripherin/rds−rom-1 complex is calculated to be 240 kDa. The protein component of this complex is estimated to be 135 kDa, providing direct evidence that the solubilized peripherin/rds−rom-1 complex is a tetramer. The abundance of this complex as measured by competitive ELISA and immunoaffinity purification is approximately 4% of total bovine ROS membrane protein and indicates that peripherin/rds−rom-1 tetramers are present at a relatively high average surface density (ca. 4100/$\mu m^{2)}$ at the rim surfaces of rod outer segment disks.

Outer segments of rod and cone photoreceptor cells are highly differentiated, membranous organelles which function in the initial stages of the visual process. They consist of over a thousand disks aligned one atop another. In rod cells, the disk membrane is spatially separated from the plasma membrane, whereas in cone cells the disk membranes are continuous with the plasma membrane. Rod outer segment disks consist of two lamellar regions which are circumscribed by a highly curved rim region. The lamellar region is densely packed with rhodopsin and serves as the surface for diffusion-mediated reactions that constitute the visual cascade system (Stryer, 1986; Liebman et al., 1987; Pugh & Lamb, 1990). The rim region, although continuous with the lamellar region, is a morphologically and biochemically distinct membrane. Chemical treatment of outer segments indicates that the rim region is less susceptible to chemical disruption than the more labile disk lamellae (Falk & Fatt, 1969). Ultrastructural studies reveal the presence of distinct filaments associated with rim regions. Such filaments appear to form connections between plasma membranes and disk rims; filaments bridging adjacent rims within disk stacks have also been documented (Usukura & Yamada, 1981; Roof & Heuser, 1982; Corless et al., 1987). Several proteins have been localized along the rim region of rod and cone outer segments including a high molecular weight "rim" protein (Papermaster et al., 1978) and two smaller membrane proteins, peripherin/rds and rom-1 (Molday et al., 1987; Bascom et al., 1992; Arikawa et al., 1992; Moritz & Molday, 1996). Although the rim region is thought to be important for stabilizing the unique outer segment structure, alternative roles in phototransduction or other outer segment processes are possible.

The disk rim specific proteins, peripherin/rds and rom-1, have been the subject of considerable interest since mutations in the genes coding for these proteins have been linked to a variety of retinal degenerative diseases including retinitis pigmentosa and macular dystrophy (Travis et al., 1989; Connell et al., 1991; Farrar et al., 1991; Kajiwara et al., 1991, 1994; Wells et al., 1993; Nicols et al., 1993a,b). Biochemical studies and primary sequence analysis have indicated that these proteins contain four putative transmembrane segments, a large intradiskal (lumenal) hydrophilic loop between the third and fourth membrane spanning segments and a relatively long C-terminal segment localized on the cytoplasmic surface of disk membranes [for review, see Molday (1994)]. Peripherin/rds and rom-1 form disulfide-linked homodimers which associate with each other to form a multisubunit complex in photoreceptor outer segment membranes (Bascom et al., 1992; Moritz & Molday, 1996) and heterologous cell expression systems (Goldberg et al., 1995). The molecular function of the peripherin/rds−rom-1 complex is not known. Several possibilities have been suggested, including morphogenesis of disk membranes, disk adhesion, stabilization of disk rim curvature, and binding of cytoskeletal elements (Molday et al., 1987; Travis et al., 1991; Arikawa et al., 1992; Molday, 1994). We have determined the quaternary structure and abundance of the peripherin/rds−

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rom-1 complex from bovine ROS[1] membranes as part of our ongoing studies to rigorously define the role of these molecules in normal photoreceptor structure and function and retinal degenerative diseases.

MATERIALS AND METHODS

Preparation and Assay of Soluble Peripherin/rds−Rom-1 Complexes. Rod outer segments (ROS) were prepared as described (Molday et al., 1987). ROS membranes were freed of cytosolic proteins by hypotonic lysis and three sequential washes in 10 mM Tris-acetate and 1 mM EDTA, pH 7.2, under dim red light. Washed ROS membranes were resuspended at 20 mg/mL in 10 mM Tris-acetate, pH 7.2, and solubilized by their dropwise addition to a 10-fold volume excess of ice-cold detergent solution containing 11 mM Tris-acetate, 110 mM NaCl, 1.1% Triton X-100, and 2.2 mM dithiothreitol (DTT). After stirring 30 min on ice, the solution was centrifuged in a Beckman TLA-45 rotor at 40K rpm (90000*g*) for 30 min at 4 °C to remove insoluble material, and the supernatant ("ROS extract") was stored at 4 °C until used. Immunoaffinity purification of the peripherin/rds−rom-1 complex, western blot analysis, and laser densitometry quantification were performed as described (Goldberg et al., 1995).

Sucrose Density Gradient Centrifugation. Sedimentation coefficient ($S_{20,w}$) and partial specific volume ($\bar{v}$) were determined according to the method of Clarke and Smigel (1989), assuming that equal amounts of detergent are bound in H_2O and D_2O. Linear gradients of 5−20% (w/w) sucrose were prepared with either H_2O or 92% D_2O containing phosphate-buffered saline (PBS), 0.1% Triton X-100 (v/v), and 2 mM DTT, pH 7.4. Samples of 100 μL, containing crude ROS extract (ca. 2 mg/mL) and marker proteins (malate dehydrogenase, aldolase, and catalase) were loaded onto gradients of 4.8 mL final volume which were prepared in 5 mL ultracentrifuge tubes. Centrifugations were performed at 4 °C for 6 h (H_2O gradients) or 12 h (D_2O gradients), in a Beckman SW 50.1 rotor spinning at 49 000 rpm. After centrifugation, tubes were punctured and 10-drop (ca. 330 μL) fractions collected and assayed for peripherin/rds−rom-1 complexes [after Goldberg et al. (1995)] and marker proteins as described below.

Gel Exclusion Chromatography. Measurement of the effective hydrodynamic (Stokes) radius of the solubilized peripherin/rds−rom-1 complex was performed by chromatography at 4 °C on a column of Sepharose Cl-6B (30 × 0.7 cm) equilibrated with PBS, 0.1% Triton X-100, and 1 mM DTT, pH 7.4. Glycerol was added to the ROS membrane extract (2 mg/mL) or purified peripherin/rds−rom-1 complexes (ca. 0.1 mg/mL) to a final concentration of 5% (v/v), and 100 μL samples were chromatographed. The void (V_o) and total (V_t) column volumes were determined with Blue Dextran and [^{35}S]ATP, respectively. Calibrating enzymes (100 μL volumes) were chromatographed individually and assayed as described below. Stokes radius (a) was determined according to the treatment of Laurent and Killander (1964).

Determination of Calibration Markers. Aldolase (4.6 nm, 7.70 S, 0.742 mL/g), catalase (5.2 nm, 11.3 S, 0.73 mL/g), β-galactosidase (15.9 S), and malate dehydrogenase (4.32 S, 0.734 mL/g) were assayed essentially as described in the *Worthington Enzyme Manual*. Thyroglobulin (8.5 nm), ferritin, and apoferritin (6.7 nm) were determined by an amido black protein assay (Schaffner & Weissmann, 1973). The physical properties of these proteins were taken from the literature (Sober, 1968; De Haën, 1987).

Estimation of Molecular Mass. Calculation of molecular mass for the detergent−protein complex was made by measurement of $S_{20,w}$, $\bar{v}$, and a (described above), and application of the Svedberg equation as presented by Siegel and Monty (1966). An estimation of molecular weight for the protein portion of the detergent−protein complex was performed by assuming additivity of partial specific volumes for detergent (0.94 mL/g,) and protein [0.74 mL/g, calculated according to Zamyatnin (1972)], after Clarke (1975).

Dissociation of the Peripherin/rds−Rom-1 Complex. ROS membrane extract was prepared as described; however, DTT was omitted from the solubilization buffer. Aliquots of the membrane extract were brought to 8 M urea and 2 M NaCl or 2 M NaI by addition of the solid reagents and stirred for 2 h at RT. Treated extracts (50 μL) were diluted 15-fold with 10 mM Tris-acetate, 100 mM NaCl, and 1% Triton X-100, immunoprecipitated with 50 μL of anti-peripherin/rds-Sepharose beads, and assayed for peripherin/rds and rom-1 binding by western blot analysis as described (Goldberg et al., 1995). Monoclonal antibody rom1D5 has been characterized previously (Moritz & Molday, 1996).

Abundance of Peripherin/rds−Rom-1 in ROS Membranes. A competitive ELISA method (Harlow & Lane, 1988) was performed to estimate the quantity of peripherin/rds−rom-1 in hypotonically washed ROS membranes. Serial dilutions of purified peripherin/rds−rom-1 (dialyzed extensively to remove peptides used during purification) or ROS membranes (50 μL) were incubated with anti-peripherin/rds monoclonal antibody per2B6 (50 μL of a tissue culture supernatant diluted 20-fold with PBS) for 1 h at RT. Aliquots of these samples (50 μL) were applied to microtiter wells containing immobilized peripherin/rds−rom-1 and assayed for antibody capture with a horseradish-peroxidase-linked secondary antibody and colorimetric detection. Polystyrene microtiter plates (96-well Nunc Polysorb) were prepared by drying 50 μL of a 200-fold dilution of ROS extract (diluted with 10 mM Tris-acetate, 100 mM NaCl, 0.1% Triton X-100) into each well overnight at 50 °C. Protein concentrations were determined with a Serva blue dye binding assay according to Read and Northcote (1981).

RESULTS

Molecular Mass of the Solubilized Peripherin/rds−Rom-1 Complex. Specialized approaches have been developed for assessing the molecular mass of integral membrane proteins in the nondenatured, detergent-solubilized state. Since substantial amounts of detergent may be bound (equal to or greater than the protein mass itself), partial specific volumes of integral membrane proteins, unlike those of soluble proteins, are found to vary significantly, thus precluding straightforward molecular mass determinations (Clarke, 1975). Therefore, a combination of techniques including gel exclusion chromatography and velocity sedimentation in

[1] Abbreviations: BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; ROS, rod outer segments; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TM4SF, transmembrane-4-superfamily; Tris, tris(hydroxymethyl)aminomethane.

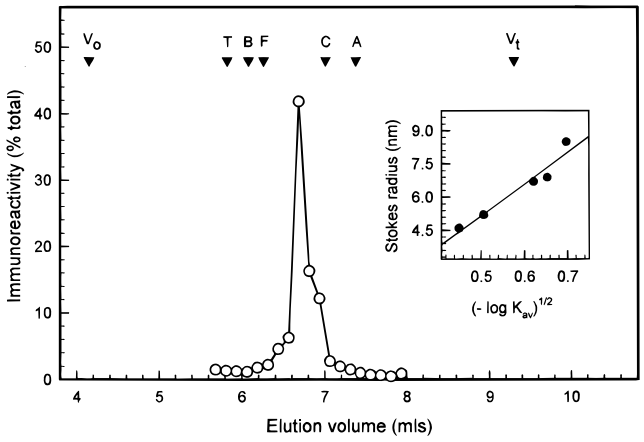

FIGURE 1: Gel exclusion chromatography of the peripherin/rds−rom-1 complex. A Triton X-100 extract of hypotonically washed ROS membranes (200 μg) was chromatographed on a Sepharose CL-6B column (30 × 0.7 cm). The peripherin/rds−rom-1 complex was detected by western blot analysis with monoclonal antibody per2B6 and quantitated by laser densitometry of films exposed to the chemiluminescent blots. Elution positions of thyroglobulin (T), β-galactosidase (B), ferritin (F), catalase (C), and aldolase (A) standard markers are indicated. (Inset) Calibration curve plots marker proteins of known Stokes radii.

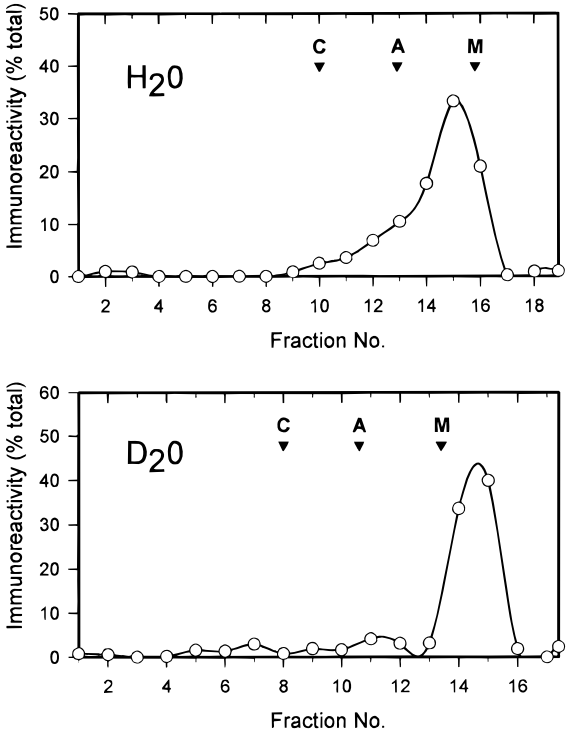

FIGURE 2: Sedimentation velocity of the peripherin/rds−rom-1 complex. A Triton X-100 extract of ROS membranes was sedimented through 5−20% sucrose gradients containing H_20 (upper panel) or 92% D_2O (lower panel). Fractions were assayed for peripherin/rds reactivity by western blot analysis with monoclonal antibody per2B6. Immunoreactivity was quantitated by laser densitometry of films exposed to the chemiluminescent blots and is plotted as percent of total immunoreactivity. Migration positions of catalase (C), aldolase (A), and malate dehydrogenase (M) are indicated.

H_2O- and D_2O-based sucrose gradients was utilized to measure the sedimentation coefficient ($S_{20,w}$), partial specific volume ($\bar{v}$), and Stokes radius (a) of the Triton X-100-solubilized peripherin/rds−rom-1 complex, in order to estimate its molecular mass.

The Stokes radius was determined by gel exclusion chromatography on a column of Sepharose Cl-6B, using well-characterized proteins as standard markers. Figure 1 shows an elution profile for a typical experiment in which Triton X-100 solubilized and immunoaffinity-purified peripherin/rds−rom-1 was chromatographed; an inset graph plots Stokes radii as a function of elution position for the calibration proteins. The results from several experiments suggest a single major species for the peripherin/rds−rom-1 complex in Triton X-100, which elutes with a Stokes radius of 6.20 ± 0.14 nm (n = 3). Similar results were obtained when chromatography was performed upon a Triton X-100 extract of ROS membranes (not shown).

The Svedberg coefficient and partial specific volume were determined by velocity sedimentation in sucrose density gradients formed in H_2O and D_2O. Figure 2 (top panel) shows a sedimentation profile for peripherin/rds−rom-1 (from a Triton X-100 extract of ROS membranes) in a water-based gradient assayed by western blot analysis and laser densitometry. A single major species is observed (S_H = 2.71 ± 0.14; n = 3), which shows a small shoulder at a slightly higher mobility. This shoulder appears to be higher-order aggregates formed by intermolecular disulfide oxidation, as it is more pronounced in the absence of reducing agents. Analogous experiments performed upon immunoaffinity-purified peripherin/rds−rom-1 gave similar results.

Sedimentation analyses were also performed for sucrose gradients in which 92% of the water was replaced by D_2O (Figure 2, lower panel). The experimental sedimentation coefficient calculated for peripherin/rds−rom-1 in D_2O (S_D = 1.15 ± 0.03; n = 4) is reduced relative to the non-detergent-binding marker proteins, indicating that this complex binds a significant amount of detergent. Indeed, the calculated partial specific volume, $\bar{v}$ = 0.83 mL/g, suggests a binding of approximately 0.8 g of Triton X-100/g of protein. This value is consistent with documented detergent-binding properties of integral membrane proteins (Clarke, 1975).

These values yield a sedimentation coefficient corrected to standard conditions ($S_{20,w}$) of 5.8 S, in reasonable agreement with a previous rough estimate of 4.5 S which assumed a partial specific volume of 0.8 mL/g and also neglected variations in experimental viscosity (Goldberg et al., 1995). The current measurements allow a calculation of a 240 kDa molecular mass for the detergent−protein complex. By assuming equivalent detergent binding in H_2O and D_2O, and additivity of partial specific volumes in the detergent-protein complex, we estimate a 135 kDa molecular mass for the detergent-free protein. Since the subunit molecular weights of peripherin/rds and rom-1 can be predicted from their primary sequences (39 and 37 kDa, respectively), we conclude that the Triton X-100 solubilized peripherin/rds−rom-1 complex is a tetramer. The hydrodynamic properties of this complex are summarized in Table 1.

Dissociation of the Peripherin/rds−Rom-1 Complex. Bascom et al. (1992) suggest that peripherin/rds and rom-1 are noncovalently associated; they observe the resolution of homodimers of these polypeptides by nonreducing SDS−PAGE. We have examined the nature of the peripherin/rds and rom-1 interaction using an independent approach. Aliquots of a ROS membrane extract were treated with 8 M urea or 2 M NaCl, in the absence of a reducing agent, diluted,

Table 1: Hydrodynamic Properties and Molecular Mass Estimate for the Peripherin/rds−Rom-1 Complex

Stokes Radius (nm)	6.2
$S_{20,w}$ (10^{-13} S)	5.8
$\bar{v}$ (mL/g)[a]	0.83
molecular mass (kDa) [detergent−protein complex]	240
detergent binding (g/g of protein)[b]	0.8
molecular mass (kDa)[c] [detergent-free protein]	135

[a] Calculated assuming equivalent detergent binding in H_2O and D_2O. [b] Estimated using the value $\bar{v} = 0.74$ mL/g for the protein portion of the complex. [c] Calculated using eq 3 from Clarke (1975).

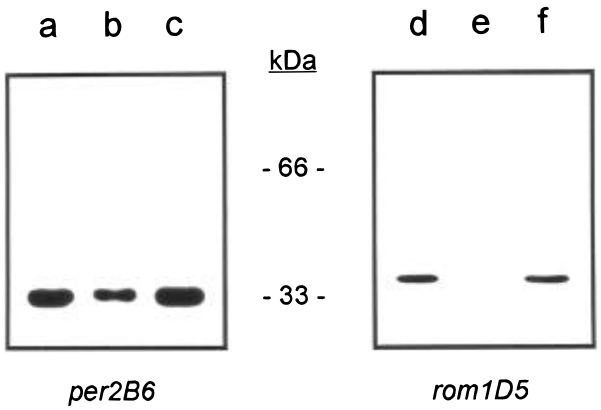

FIGURE 3: Disruption of the peripherin/rds−rom-1 interaction. Aliquots of Triton X-100 solubilized ROS membranes were either left untreated as controls (lanes a and d) or were treated with 8 M urea (lanes b and e) or 2 M NaCl (lanes c and f) prior to dilution and immunoprecipitation with per2B6-Sepharose. Immunoprecipitates were analyzed by western blot analysis with anti-peripherin/rds (per2B6, left panel) and anti-rom-1 (rom1D5, right panel) monoclonal antibodies. Rom-1 coprecipitated in the control and NaCl treated samples but was dissociated by treatment with urea.

and then immunoprecipitated with a monoclonal antibody directed against peripherin/rds (per2B6). Figure 3 shows that, in each instance, peripherin/rds reactivity is associated with the immunoaffinity matrix (left panel, lanes a, b, and c). In the untreated control, rom-1 coprecipitates with peripherin/rds (right panel, lane d), reflecting its tight association [documented previously in Bascom et al. (1992) and Moritz and Molday (1996)]. In contrast, rom-1 does not coprecipitate in urea treated samples, indicating that dissociation from peripherin/rds has occurred (lane e). Dissociation is also promoted by the strong chaotrope NaI (not shown) but is not observed for samples treated with 2 M NaCl (lane f). Nonreducing SDS−PAGE of dissociated samples indicate that each protein retains its homodimeric form (not shown). These results demonstrate that peripherin/rds can be dissociated from rom-1 under nonreducing conditions; however, strong denaturants or chaotropic agents are required.

Abundance of the Peripherin/rds−Rom-1 Complex in ROS Membranes. The concentration of the peripherin/rds−rom-1 complex in extracts of washed ROS membranes was examined by competitive ELISA. This approach characterizes the quantity of protein required for half-maximal inhibition of antibody capture by an antigen immobilized to a solid support. Figure 4 shows the results obtained for anti-peripherin/rds monoclonal antibody per2B6 binding to Triton X-100 solubilized ROS membranes immobilized on polystyrene microtiter plates. Both purified peripherin/rds−rom-1 and solubilized ROS membranes inhibited antibody binding to the solid phase (Figure 4); average half-maximal

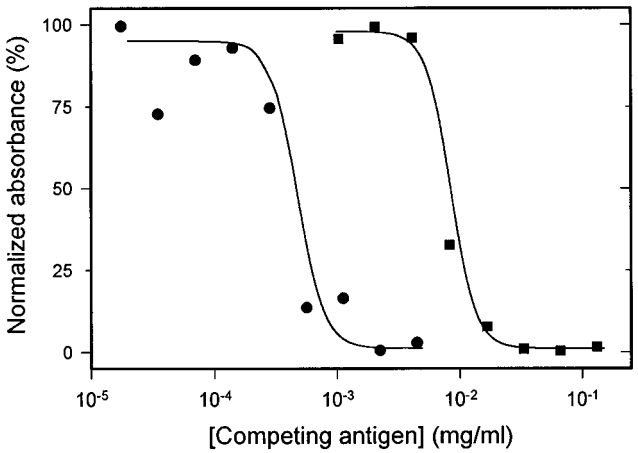

FIGURE 4: Abundance of peripherin/rds−rom-1 complex in bovine ROS. ELISA assays measuring the competitive inhibition of anti-peripherin/rds monoclonal antibody per2B6 binding to solubilized ROS membranes immobilized on a microtiter plate. A fixed concentration of antibody was preincubated with serial dilutions of purified peripherin/rds−rom-1 complex (●) or hypotonically washed and solubilized ROS membranes (■) prior to colorimetric assay in microtiter plates. Points are averaged triplicate determinations.

inhibition concentrations were 0.41×10^{-3} and 6.42×10^{-3} mg/mL, respectively (mean of two experiments). The ratio of these values suggests that the peripherin/rds−rom-1 complex constitutes 6% of total ROS membrane protein. By way of comparison, immunoaffinity purification of this complex routinely indicates an abundance of ca. 1−2% of total ROS membrane protein. Thus, the peripherin/rds−rom-1 complex comprises roughly 4% of the total protein of the bovine ROS membrane; given a rhodopsin content of 90% (Papermaster & Dreyer, 1974), this corresponds to a molar ratio of peripherin/rds−rom-1 to rhodopsin of ca. 1:90.

DISCUSSION

Using a hydrodynamic analysis, we have estimated a molecular mass of 135 kDa for the detergent-free peripherin/rds−rom-1 protein complex. The determination of molecular weight for integral membrane proteins in the detergent solubilized native state is not a trivial problem. The value we obtain is subject to uncertainty arising primarily from assumptions made regarding detergent binding. Clarke (1975) has shown that solvent-dependent differences in binding of detergent do occur, and, in general, less detergent is bound in D_2O (compared to H_2O), resulting in underestimates of molecular mass by 5−10%. An error of this sign and magnitude would increase our estimate to approximately 140−150 kDa. Since the deduced primary sequences of peripherin/rds and rom-1 predict polypeptides of 39 and 37 kDa, respectively (Connell & Molday, 1990; Bascom et al., 1992), we conclude that the Tritron-X-100 solubilized peripherin/rds−rom-1 complex consists of four polypeptide subunits having a molecular mass of approximately 152 kDa. We consider it likely that this tetrameric form is the minimal structural unit required for function at outer segments disk rims.

A single major hydrodynamic species was observed in all experiments. More rapidly sedimenting species were observed in the absence of reducing agents or as a function of time in detergent solution. We attribute these higher order aggregates to secondary oxidation processes and the general tendency of integral membrane proteins to self-associate in

detergent solution; however, it is possible that self-association of tetramers into higher order structures does occur within disk membranes. The observation that purification of peripherin/rds−rom-1 does not influence its sedimentation mobility is a strong indication that associations with other ROS components are not present (or maintained) in the Triton-solubilized state. These observations do not preclude the existence of such interactions *in situ*; although nonionic detergents such as Triton X-100 are relatively gentle, protein structure and function are sometimes affected by their use (Neugebauer, 1990).

Previous studies indicate that peripherin/rds and rom-1 are assembled as disulfide-linked homodimers, both in ROS membranes (Connell & Molday, 1990; Travis et al., 1991; Bascom et al., 1992) and when heterologously expressed in COS-1 cells (Goldberg et al., 1995). Two-dimensional (nonreducing/reducing) SDS−PAGE has suggested that peripherin/rds and rom-1 homodimers are associated through noncovalent interactions (Bascom et al., 1992). We confirm that it is possible to dissociate peripherin/rds from rom-1 under nonreducing conditions in the presence of strong denaturants or chaotropic agents; however, their interaction is not disrupted by increased ionic strength. These results suggest a noncovalent association which involves a significant hydrophobic component, possibly involving transmembrane regions of the polypeptides. These observations are consistent with the previously proposed view that peripherin/rds and rom-1 are actually subunits of a single functional unit, rather than functionally independent proteins which can reversibly associate (Goldberg et al., 1995).

We have measured the abundance of the peripherin/rds−rom-1 complex in bovine ROS and conclude that it constitutes roughly 4% of total ROS membrane protein, a ratio of one tetrameric complex for each 90 rhodopsin molecules present. Since the tetramers are confined to a very limited portion of the ROS disc membrane, their average surface density is expected to be rather high. Assuming an average disk diameter of 1.25 μm and a rhodopsin density of 25 000/μm^2, each bovine disk is predicted to contain some 700 peripherin/rds−rom-1 tetramers. By modeling a disk rim as a torus (1.218 μm inner diameter, 1.25 μm outer diameter) intersected by a 12 nm thick (planar) lamellar region which contains a single incisure (extending 30% across the disk diameter), we calculate an average rim surface density of ca. 4100/μm^2. This relatively high density can be compared to other integral membrane proteins localized to the ROS plasma membrane: ca. 300/μm^2 for the cGMP-gated cation channel (Cook et al., 1989) and ca. 600/μm^2 for the Na^+/Ca^{2+}-K^+ exchanger (Cook & Kaupp, 1988).

Previous ultrastructural studies of photoreceptor disks have documented rim-localized features which are currently undefined at the molecular level (Usukura & Yamada, 1981; Roof & Heuser, 1982; Corless et al., 1987). Filamentous structures regularly spaced at 14−19 nm intervals have been observed to connect rim regions (margins and incisures) of adjacent disks in amphibian and bovine ROS and are presumed responsible for the structural integrity of disk stacks (Roof & Heuser, 1982; Corless et al., 1987). If 700 peripherin/rds−rom-1 complexes are distributed uniformly along the circumference and incisure of the model bovine disk rim region (described above), a minimum spacing of ca. 7 nm is calculated. These values indicate that peripherin/rds−rom-1 tetrameric complexes are present in outer segment disks in quantities similar to the numbers of disk−disk filaments documented previously; additional studies are required to assess the precise arrangement of peripherin/rds−rom-1 tetramers at disk rims and their potential involvement in interdiskal filament structure and function.

Alternative (or additional) roles for the peripherin/rds−rom-1 complex are also possible. These photoreceptor proteins show some structural similarities to a recently described cell surface protein from platelets (Roberts et al., 1995), a member of the transmembrane-4-superfamily (abbreviated TM4SF; Wright & Tomlinson, 1991). Although the peripherin/rds and rom-1 protein sequences display limited homology to other superfamily members, the conservation of predicted topology, orientation, and cysteine residues in each protein suggests that the photoreceptor proteins may indeed be evolutionarily related molecules. The region of highest homology (ca. 10% identity) exists in the large extracellular/intradiskal loop which links transmembrane segments M3 and M4, implying a conservation of function in this region, although that function remains obscure. It has been suggested that the members of the TM4SF are involved in signal transduction (Wright & Tomlinson, 1991). Indeed, Kemp et al. (1994) have shown that mutations in peripherin/rds can lead to slowed dark adaptation in degenerating human retinas. We consider it plausible that, in addition to acting as a structural element in outer segment morphogenesis, peripherin/rds−rom-1 could be involved in an as yet undefined signal transduction process.

In summary, we have defined the normal quaternary structure of the solubilized peripherin/rds−rom-1 rim complex as tetrameric; disulfide-linked homodimers of peripherin/rds and rom-1 interact noncovalently to form minimal functional units. This tetrameric complex represents approximately 4% of total ROS membrane protein, indicating a relatively high average surface density at disk rim regions.

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