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Structural and functional properties of rhodopsin from rod outer segment disk and plasma membrane

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The structural and functional properties of bovine rhodopsin from rod outer segment disk and plasma membranes were compared by high performance liquid chromatography (HPLC), mass spectrometric analyses, and in vitro rhodopsin phosphorylation assays. Disk and plasma membranes separated by a ricin gold-dextran affinity perturbation method were treated with trypsin or cyanogen bromide, and the N-terminal and C-terminal rhodopsin peptides were isolated by immunoaffinity chromatography using antirhodopsin monoclonal antibodies coupled to Sepharose. Reverse phase HPLC chromatograms of the C-terminal and N-terminal peptides from disk and plasma membrane rhodopsin were found to be similar. Mass spectrometric, PicoTag, and hexose analyses of the tryptic 1–16 N-terminal peptides further indicated that the post-translational glycosylation of plasma membrane rhodopsin is identical to that of disk membrane rhodopsin. HPLC analysis of soluble peptides obtained from cyanogen bromide and tryptic digestion of immunoaffinity purified rhodopsin also indicated that no significant differences exist between disk and plasma membrane rhodopsin. Light-induced phosphorylation of rhodopsin in disk and plasma membranes were also compared using in vitro phosphorylation assays. Plasma membrane rhodopsin was found to undergo light-dependent, rhodopsin kinase catalyzed phosphorylation to the same extent as disk membrane rhodopsin. These results indicate that the bulk rhodopsin in rod outer segment plasma membranes appears to be identical to rhodopsin in disk membranes in regard to primary structure, post-translational glycosylation and light-dependent phosphorylation. On this basis, it is unlikely that the sorting of rhodopsin between disk and plasma membranes occurs by a mechanism based on differences in structural properties of rhodopsin.

Introduction

The rod outer segment (ROS) is a specialized compartment of the vertebrate rod photoreceptor cell which functions in the absorption of light and its transduction into electrical signals. Each ROS consists of an ordered stack of closed disks surrounded by a separate plasma membrane over most of the length of the outer segment. At the base of the outer segment, however, evagination of the ciliary plasma membrane produces continuously folded 'nascent' disk membranes from which mature disks and the plasma membrane form [1].

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Disk and plasma membranes separated by affinity density perturbation methods have been shown to have different protein and lipid compositions [2–4]. The plasma membrane contains the 63 kDa cGMP-gated cation channel and an associated 240 kDa spectrin-like protein [5,6], the Na^+/Ca^{2+}-K^+ exchanger [7], membrane-associated glyceraldehyde-3-phosphate dehydrogenase [8], GLUT-1 glucose transporter [9], and other unidentified proteins [3]. Disk membranes contain a high molecular weight rim protein [3,10], the peripherin/*rds* protein [11], ROM-1 protein [12] and several other proteins [3]. The lipid composition of the plasma membrane has been reported to have a high content of cholesterol, unsaturated C_{18} (18:2; 18:3) fatty acids, and the saturated C_{14} fatty acid, whereas the disk membranes are high in the saturated C_{18} fatty acid and unsaturated C_{22} (22:6) docosahexaenoic acid [4].

Despite these differences in lipid and protein compositions, both the disk and plasma membrane have a high concentration of the photoreceptor protein rhodopsin [2,3,13]. Rhodopsin from disk membranes

has been extensively studied [14,15]. The polypeptide contains 348 amino acids with an 11-*cis*-retinal chromophore covalently linked to lysine-296. The polypeptide appears to span the disk membrane seven times with the N-terminus oriented towards the intradiskal side of the disk membrane and the extracellular side of the plasma membrane and the C-terminus exposed on the cytoplasmic surface. The N-terminus contains two N-linked carbohydrate chains at asparagine residues 2 and 15 [16,17], and the C-terminus contains seven serine and threonine residues which can be phosphorylated by rhodopsin kinase upon the photobleaching of rhodopsin [18]. Two adjacent cysteine residues (Cys-322 and Cys-323) near the C-terminus contain thioester linked palmitate residues [19,20].

Although much is known about the structural and functional properties of disk membrane rhodopsin, little is known about the properties of the less abundant plasma membrane rhodopsin. In particular, it is of interest to know if rhodopsin in disk and plasma membrane undergo differential post-translational modification which may serve to selectively sort rhodopsin to either the disk or plasma membrane during ROS morphogenesis. In this paper, we compare some of the structural and functional properties of rhodopsin isolated from disk and plasma membranes.

Materials and Methods

Preparation of rod outer segment membranes

ROS were isolated from retinas of freshly dissected bovine eyes by sucrose gradient density centrifugation, and the ROS disk and plasma membranes were separated by the ricin-gold-dextran affinity density perturbation techniques as previously described [3].

SDS gel electrophoresis and Western blotting

SDS gel electrophoresis was carried out on 8% polyacrylamide slab gels using the buffer system of Laemmli [21]. Approx. 40–60 μg of membrane proteins were loaded onto each lane. Gel slices were either stained with Coomassie Blue R250 or electroblotted onto Immobilon membrane (Millipore) for Western blotting [3]. The blots were blocked with 0.5% Tween 20 in phosphate-buffered saline (PBS) and subsequently labeled with 1:20 diluted hybridoma culture supernatant containing the PMc 1D1 monoclonal antibody against the cGMP-gated channel [5] for 1 h, washed with PBS containing 0.05% Tween 20 and relabeled with ^{125}I-labeled goat anti-mouse Ig for detection by autoradiography.

Preparation of anti-rhodopsin immunoaffinity columns

Antirhodopsin monoclonal antibodies rho 1D4 [22] and rho 4D2 [23] were purified from ascites fluids by ammonium sulphate precipitation and DEAE anion exchange chromatography and coupled to CNBr activated Sepharose CL-2B beads at a concentration of 1.5–2 mg/ml as previously described [24].

Purification of N-terminal and C-terminal fragments of rhodopsin

The amino-terminal and carboxyl-terminal fragments of disk and plasma membrane rhodopsin were generated by either cyanogen bromide or tryptic digestion as modified from published procedures [25,26]. For the CNBr cleavage reaction, 1 mg (protein) of disk membranes and 2 mg of plasma membrane were washed twice with distilled water by centrifugation at 15000 rpm for 20 min in a Sorvall SS-34 rotor and solubilized in 70% formic acid (1 ml/10 mg protein). Cyanogen bromide was then added (5 mg CNBr/mg protein), and the cleavage was allowed to proceed for 14–17 h in the dark at 23°C. The digest was dried down using a Savant Speed Vac concentrator, and soluble peptides were extracted from the pellet with 10 mM ammonium bicarbonate at pH 7.8. The extracted peptides were loaded onto a 3 ml rho 1D4 or rho 4D2 Sepharose CL-2B column pre-equilibrated in 50 mM ammonium bicarbonate (pH 7.8). The column was washed with five column volumes of the above buffer and the bound peptides were eluted with three column volumes of 0.1 M acetic acid. The acid eluants were dried down and resuspended in 220 μl of distilled water containing 0.05% trifluoroacetic acid (TFA) for HPLC analysis. For tryptic digestion, 1 mg of disk membrane proteins or 2 mg of plasma membrane proteins were washed twice with 10 mM ammonium bicarbonate (pH 7.8) and solubilized in the same buffer containing 18 mM 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate (CHAPS) detergent at a concentration of 1 mg protein/ml. Trypsin was then added (0.1 mg/ml) and cleavage reaction was carried out overnight at 23°C. The digest was first passed through 1 ml of ovomucoid-Sepharose CL-2B column (1.0 mg ovomucoid/ml packed beads) to remove the trypsin and then loaded onto a 2–3 ml rho 1D4 or rho 4D2 immunoaffinity column pre-equilibrated in the solubilization buffer. The column was washed with two column volumes of the solubilization buffer and then with five column volumes of 50 mM ammonium bicarbonate (pH 7.8). The bound peptides were then eluted from the columns with three column volumes of 0.1 M acetic acid and prepared for HPLC analysis as above. The recovery of rhodopsin peptides was approx. 70% for both disk and plasma membranes.

Purification of rhodopsin from disk and plasma membranes for peptide mapping studies

ROS plasma membranes (8 mg protein) or disk membranes (2.5 mg protein) were solubilized in 18 mM CHAPS in PBS at a concentration of 1 mg protein/ml.

The solution was centrifuged at 12000 rpm for 30 min in a Sorvall SS 34 rotor to remove unsolubilized material, and the supernatant was applied to a 4 ml rho 1D4 antibody-Sepharose column equilibrated in the solubilization buffer. The column was then washed with five column volumes of the same buffer, and rhodopsin was eluted with 10 mM CHAPS in PBS containing 25 μg/ml of synthetic *N*-acetylated peptide corresponding to the C-terminus of rhodopsin (Ac-$_{340}$Thr-Glu-Thr-Ser-Gln-Val-Ala-Pro-$_{348}$Ala-COOH). The peptide-eluted fractions containing rhodopsin were pooled and dialyzed against distilled water for 4 days with five changes of water to remove the peptide. The precipitated rhodopsin was collected by centrifugation and was subjected to either tryptic or CNBr cleavage. Approx. 50% of rhodopsin was recovered after dialysis. Tryptic digestion was carried out by resuspending 0.5–1 mg of the precipitated rhodopsin in 300 μl of 25 mM ammonium bicarbonate buffer (pH 7.8) in the presence of 25 μg/ml of trypsin. The digestion was allowed to proceed for 48 h. After spinning down the insoluble rhodopsin fragments, the supernatant was dried down and resuspended in 220 μl of distilled water containing 0.05% TFA for HPLC analysis. The CNBr cleavage of 0.5–1 mg rhodopsin was carried out as described above.

Analysis of rhodopsin peptides by HPLC

Tryptic and CNBr-cleaved peptides (200 μl) were analyzed by reverse phase HPLC using a 3.9 × 300 mm μ BONDAPAK C_{18} (Waters) column with a 5–40% acetonitrile gradient containing 0.05% TFA. No peptide peaks were observed after 40% acetonitrile.

Analysis of rhodopsin N-terminal peptides by mass spectrometry, PicoTag and hexose analyses

Tryptic rhodopsin peptides purified by rho 4D2 affinity chromatography and HPLC chromatography from 1 mg of disk and 2 mg of plasma membranes were dried down and resuspended in 100 μl deionized, distilled water. Mass spectrometry was carried out using a Finnigan Lasermat laser desorption mass spectrometer fitted with a 337 nm nitrogen laser and controlled by the manufacturer's software running on a Compaq Deskpro 386/20e computer. The laser energy used was just sufficient to ionize the sample. The sample (0.1 μl) and matrix (0.5 μl of 10 mg/ml 2,5-dihydroxybenzoic acid dissolved in 1:4 acetonitrile/water) were applied to standard manufacturer's targets and allowed to dry thoroughly before being analyzed. The ratio of matrix to sample was typically 1000:1.

Amino acid and hexosamine analysis was carried out as previously described [27]. Briefly, 5 μl of the peptides were hydrolyzed in vapour phase HCl for 6 h at 100°C for hexosamine analysis or for 24 h at 112°C for amino acid composition. Derivatization with phenylisothiocyanate was carried out by the Waters PicoTag method according to the manufacturer's protocols. The resultant phenylthiocarbamyl derivatives were separated by reverse phase HPLC on a Hichrom 5 μm Spherisorb ODS2 column (0.46 × 25 cm) at 50°C using modifications of the Waters PicoTag solvent and gradient system.

The hexose analysis was performed according to the method of Honda et al. [28]. Mannose, rhamnose, glucose, galactose and fucose were used as standards. 25 nmol of standards and 5 μl of glycopeptides were hydrolyzed, derivatized, and resuspended in 200 μl of HPLC buffer. 10 μl of the derivatized standards and samples were injected separately into a HPLC column (Hichrom RPB 4.6 mm × 25 cm).

In vitro phosphorylation of disk and plasma membrane rhodopsin

Neuraminidase treated ROS membranes labeled with ricin-gold-dextran were phosphorylated by adding 3 μl of [γ-^{32}P]ATP (10 μCi/μl) to 3 ml of ROS membranes (8–10 mg protein) in buffer A (0.05 M KH_2PO_4 (pH 7.0), 2 mM $MgCl_2$, 1 mM DTT and 3 mM ATP) under dim red light. The membranes were then bleached under continuous white light for 30 min at 23°C. As a control, the phosphorylation reaction was also performed without bleaching. Phosphorylation reactions were stopped by washing the membranes four times with 10 ml of 10 mM Tris (pH 7.2), containing 2 mM EDTA. Disk and plasma membranes were dissociated with trypsin and separated by density perturbation method [3]. The purified disk and plasma membranes were resuspended in approx. 150 μl of 10 mM Tris buffer (pH 7.2). 50 μl of the disk and plasma membranes were solubilized in 2 ml of 0.2% Triton X-100 in PBS, and the solution was centrifuged at 12000 rpm for 30 min. A 0.5 ml aliquot of the supernatant was added to 8 ml of Aquasol II in duplicate and counted in a liquid scintillation counter to determine the extent of phosphorylation. The amount of rhodopsin in the membrane samples was determined by a solid phase competitive radioimmune assay (RIA) using immunoaffinity column purified rhodopsin as a standard. The competitive RIA was carried out with rho 4D2 culture supernatant as described previously [22].

Results

Analysis of the disk and plasma membrane fractions

The purity of the bovine ROS disk and plasma membrane isolated by the ricin-gold-dextran affinity density perturbation method was determined by SDS gel electrophoresis and Western blotting. As shown in Fig. 1, the disk membrane fraction contained rhodopsin as the major membrane protein and the trypsin digested form of the 220 kDa rim protein (120 kDa fragment) as a prominent band [3]. The ROS plasma

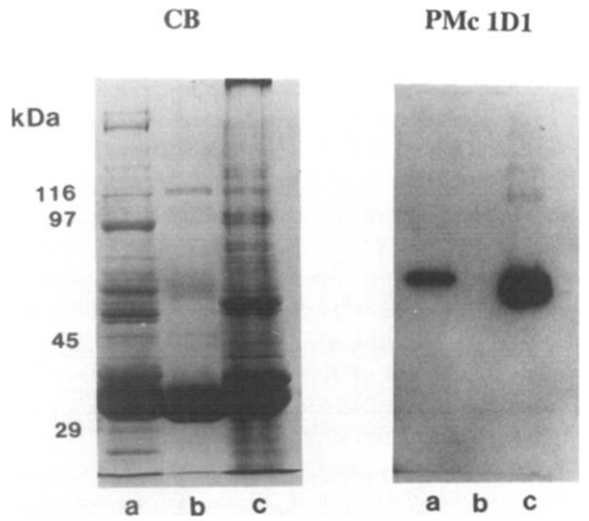

Fig. 1. SDS polyacrylamide gel electrophoresis and Western blotting analysis of the ROS membrane fractions. Purified ROS (lane a), disk membranes (lane b) and plasma membrane (lane c) containing 40–60 μg of protein were loaded on a 8% SDS polyacrylamide slab gel. Gels were either stained with Coomassie blue (CB) or electrophoretically transferred to Immobilon membranes and labeled with the anti-cGMP-gated channel monoclonal antibody (PMc 1D1) and ^{125}I-labeled goat anti-mouse Ig for autoradiography.

membrane contained rhodopsin and the membrane associated form of glyceraldehyde-3-phosphate dehydrogenase (apparent molecular weight 38000) as the most prominent proteins [8], and an intense band migrating with an apparent molecular weight of 59000. The purity of the membrane preparations was further assessed by Western blotting with the anti-cGMP-gated channel monoclonal antibody PMc 1D1. As shown in Fig. 1, both the intact and trypsinized forms of the channel (approx. 63 kDa and 59 kDa, respectively) were intensely labeled with the PMc 1D1 antibody in the ROS plasma membrane, but not in the disk membrane [5]. This indicated that the disk fraction is devoid of plasma membrane contamination. The purity of the plasma membrane fraction was determined by competetive ELISA using a disk specific anti-peripherin 2B6 monoclonal antibody [3,11]. From determination of the protein concentration required to attain half-maximum inhibition, the plasma membrane was estimated to contain less than 23% of the amount of peripherin found in disk membranes. If one assumes that peripherin is only present in disk membranes, then the plasma membrane can be considered over 77% pure. Assuming that 90% of disk membrane proteins is rhodopsin and that rhodopsin makes up 50% of plasma membrane proteins, then the plasma membrane preparation will contain less than 35% of the rhodopsin that is derived from disks. Thus, majority of the rhodopsin peptides will originate from plasma membrane rhodopsin and differences if present should be readily detectable.

HPLC analysis of N-terminal and C-terminal rhodopsin peptides

Since the N-terminus of rhodopsin contains the two sites of *N*-linked glycosylation, this segment was considered as a candidate for differences in post-translational modification of disk and plasma membrane rhodopsin. This was investigated by subjecting isolated disk membranes and isolated plasma membranes to either trypsin digestion or CNBr cleavage. The N-terminal rhodopsin peptides were then affinity purified on a rho 4D2 monoclonal antibody-Sepharose column and analyzed by reverse phase HPLC. As shown in Fig. 2, the elution profiles and retention times for these N-terminal rhodopsin peptides from disk and plasma membranes were the same. To determine if the carbohydrates of disk and plasma membrane rhodopsin differ, tryptic N-terminal fragments purified by affinity chromatography and HPLC chromatography were examined by laser desorption mass spectrometry. As shown in Fig. 3, the spectra of the N-terminal tryptic fragments of disk and plasma membrane rhodopsin were the same. The main signal represented a relative mass of approx. 4060 which corresponds to the combined molecular weights of the peptide + 6 *N*-acetylglucosamines + 6 mannoses + 1 Na ion adduct.

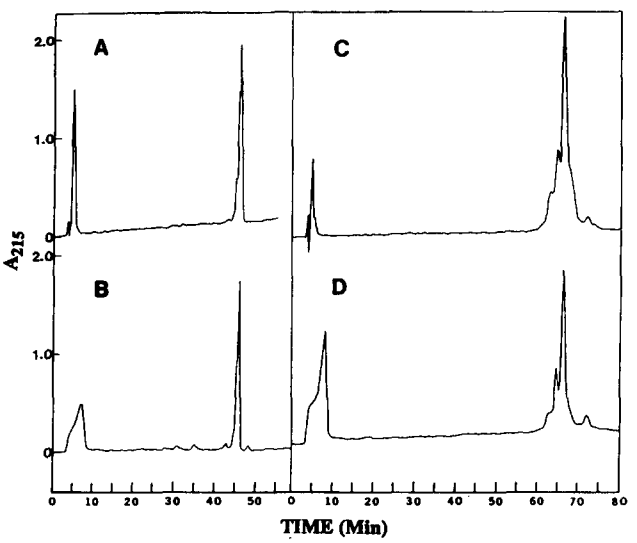

Fig. 2. HPLC chromatographs of tryptic N-terminal 1–16 and CNBr N-terminal 2–39 amino acid peptides from ROS disk (A and C) and plasma membrane (B and D) rhodopsin respectively. 1 mg of disk membrane protein and 2 mg of plasma membrane protein were subjected to either tryptic or CNBr cleavages. The N-terminal peptides were purified on a rho 4D2 anti-rhodopsin antibody-Sepharose column and analyzed by reverse-phase HPLC using an acetonitrile gradient. The absorbance of the peptide peaks was monitored at 215 nm. The peaks at 5 min are the injection peaks.

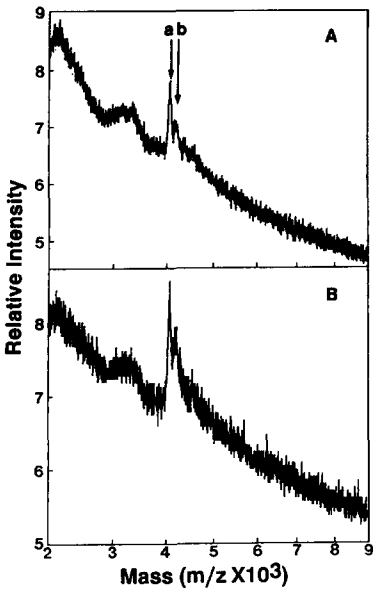

Fig. 3. Mass spectrometic analysis of tryptic N-terminal peptides from ROS disk (A) and plasma membrane (B). Tryptic rhodopsin N-terminal fragments from disk and plasma membranes were purified by rho 4D2 antibody-Sepharose columns and reverse phase HPLC. The peptides were then analyzed by laser desorption mass spectrometry. Peak a and peak b correspond to a relative mass of 4060 and 4214, respectively.

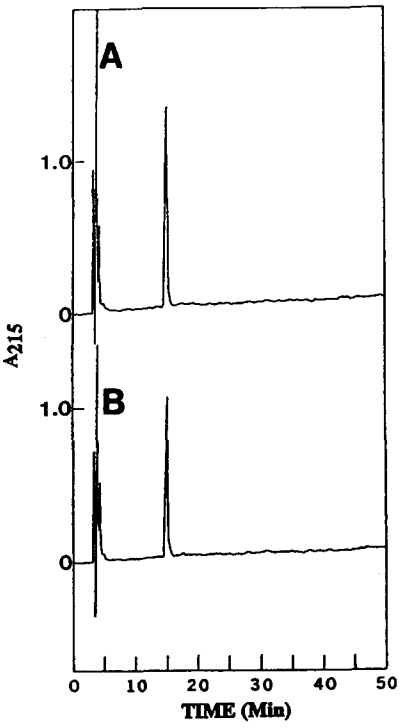

Fig. 4. HPLC chromatographs of tryptic C-terminal 340–348 amino acid peptide from disk (A) and plasma membrane (B) rhodopsin. 1 mg of disk membrane proteins and 2 mg of plasma membrane proteins were digested with trypsin. The C-terminal fragments were purified on a rho 1D4 anti-rhodopsin antibody-Sepharose column and analyzed by reverse phase HPLC.

This is in agreement with earlier studies [17,29] indicating that the major carbohydrate species of bovine rhodopsin consists of $(Man)_3(GlcNAc)_3$. The next major peak with an average mass of 4214 was in close agreement to the molecular weight of the peptide + $1(Man)_4(GlcNAc)_3$ + $1(Man)_3(GlcNAc)_3$ + 1 Na ion adduct (calculated molecular weight = 4219). Only a minor signal characteristic of a rhodopsin glycopeptide containing $(Man)_5(GlcNAc)_3$ was observed. The mass of a Na ion is included because underivatized free or peptide bound oligosaccharides in 2,5-dihydroxybenzoic acid matrix always tend to ionize and pair with sodium rather than to be present in a protonated form. The intensities of the glycopeptides were very low compared to the baseline due to the large amount of matrix material and ions present. The N-terminal tryptic disk and plasma membrane rhodopsin peptides were found to be similar in terms of amino acid and hexosamine content by PicoTag analysis. In addition, galactose was not detected in either of the samples by hexose composition analysis.

The C-terminus of rhodopsin which serves as the primary site of phosphorylation by rhodopsin kinase was analyzed by a similar procedure. Tryptic C-terminal fragment of rhodopsin from either disk or plasma membranes was purified on a rho 1D4 monoclonal antibody-Sepharose column and analyzed by HPLC chromatography. Fig. 4 indicates that the same C-terminal fragment is generated by trypsin digestion of disk and plasma membranes. This fragment has been shown to consist of amino acids 340–348 of the rhodopsin sequence [22].

HPLC analysis of disk and plasma membrane rhodopsin peptides

In order to determine if other segments of rhodopsin are differentially modified, rhodopsin from detergent solubilized disk and plasma membrane was purified by immunoadsorption to a rho 1D4 antibody-Sepharose column and selective elution with a synthetic 9 amino

TABLE I

Light-induced phosphorylation of rhodopsin from ROS disk and plasma membranes

	Light			Dark		
	dpm [a]	μg rho [a]	n [b]	dpm [a]	μg rho [a]	n [b]
Disks	24218	75.0	1.5±0.1	222	52.5	0
P.M.	3237	10.3	1.7±0.2	100	12.5	0

[a] Data from one of the two phosphorylation assays showing the extent of ^{32}P incorporation in dpm; rhodopsin was determined by competitive RIA using rho 4D2 monoclonal antibody.

[b] The number of mol of phosphate incorporated into each mol of rhodopsin (n) was calculated from two phosphorylation assays.

acid peptide corresponding to the C-terminus of rhodopsin. After extensive dialysis to remove the detergent and the synthetic peptide, the purified rhodopsin was subjected to trypsin or CNBr cleavage for analysis of soluble peptides by HPLC. The HPLC profiles of soluble peptides from tryptic and CNBr digests are shown in Fig. 5. Peptide maps of disk and plasma membrane rhodopsin were similar. The number of peaks observed, four for tryptic peptides and seven for the CNBr peptides, is lower than the number of peptides predicted from the rhodopsin sequence (17 tryptic peptides and 15 CNBr peptides). This is due to the insolubility of many of the rhodopsin peptides. The peaks corresponding to the N-terminal peptide and the C-terminal peptide were identified on the basis of comparison with the HPLC chromatograms of the purified peptides in Figs. 2 and 4.

Light-induced phosphorylation of ROS disk and plasma membrane rhodopsin

The extent to which rhodopsin in disk and plasma membranes undergoes light-induced, rhodopsin kinase catalyzed phosphorylation was investigated. In this study, [γ-^{32}P]ATP phosphorylation of rhodopsin in ROS was initiated by illumination, and the ROS disk and plasma membranes were separated by affinity density perturbation. As shown in Table I, the extent of phosphate incorporation into bleached rhodopsin in disk and plasma membranes was similar.

Discussion

Previous studies have shown that bulk rhodopsin from disk membranes undergoes post-translational modification at both the N-terminal and C-terminal regions. The N-terminus of disk membrane rhodopsin is acetylated [20], and Asn residues at positions 2 and 15 are glycosylated with oligosaccharide chains consisting of *N*-acetylglucosamine and mannose residues [17,29,30]. The carboxyl-terminal segment undergoes acylation at Cys-322 and 323 with palmitate [19,20], and phosphorylation at multiple serine and threonine residues by a light dependent rhodopsin kinase catalyzed reaction [18]. In order to determine if plasma membrane rhodopsin undergoes similar modifications, the amino and carboxyl-terminal peptides of disk and plasma membrane rhodopsin were analyzed. The HPLC chromatograms of the 2–39 CNBr and 1–16 tryptic N-terminal peptides and mass spectrometric analysis of the 1–16 tryptic N-terminal peptides from disk membrane rhodopsin were identical to the corresponding peptides derived from plasma membrane rhodopsin. This indicates that no major differences in the N-terminal segment or oligosaccharide chains exist. The multiple peaks observed in the HPLC chromatograms and mass spectrometric spectra are most likely due to carbohydrate heterogeneity [17,29,30]. Metabolic labeling studies indicate that a small amount of galactose is transiently incorporated into rhodopsin [31] and this

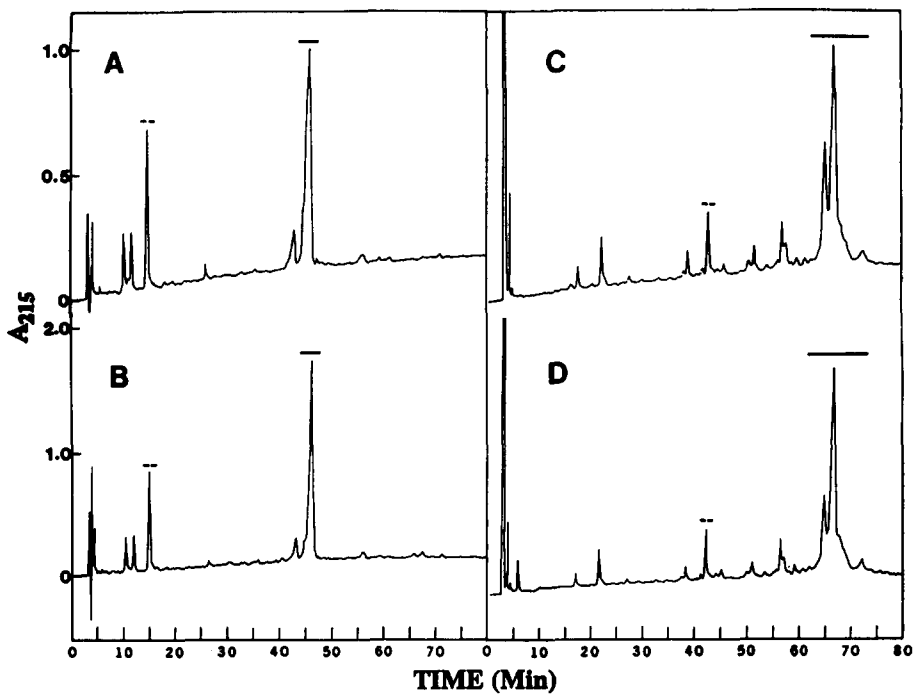

Fig. 5. HPLC chromatographs of tryptic and CNBr peptides from disk (A and C) and plasma membrane (B and D) rhodopsin. Disk membrane (0.5 mg protein) and plasma membrane (1 mg protein) rhodopsin were purified on a rho 1D4 antibody-Sepharose column and subjected to either tryptic or CNBr digestion. The soluble rhodopsin peptides were analyzed by reverse phase HPLC using an acetonitrile gradient. The tryptic and CNBr C-terminal (--) and N-terminal peptides (———) of rhodopsin are indicated.

incorporation is preferentially localized to the plasma membrane rhodopsin [32]. In this study, galactose was not detected in either the disk or plasma membrane rhodopsin. It is possible that the amount of galactose present in bovine rhodopsin is below the level of detection. Alternatively, galactose is not incorporated into rhodopsin as previously reported [31,32], but is incorporated into a minor glycoprotein of the same subunit molecular weight which copurifies with rhodopsin. In this regard, peripherin/rds has been shown to be a glycoprotein which migrates with rhodopsin by SDS gel electrophoresis [11,34], and which can associate with rhodopsin under some conditions [12].

The C-terminus of disk and plasma membrane rhodopsin also appears to be identical as analyzed by HPLC analysis of the 9 amino acid tryptic peptide fragment. Although attempts to immunoaffinity purify the longer CNBr C-terminal rhodopsin peptide were unsuccessful possibly due to the acylated cysteine residues, the CNBr C-terminal rhodopsin peptide was identified from HPLC fractionated CNBr-cleaved rhodopsin by radioimmune assays. The similarity in the retention time of this 31 amino acid fragment from disk and plasma membrane rhodopsin supported the view that no differences in post-translational modification at the C-terminus are present between disk and plasma membrane rhodopsin.

Finally, soluble peptides from immunoaffinity purified disk and plasma membrane rhodopsin were fractionated on a reverse phase HPLC column. Similarity in the elution profiles and retention times of the peptide fragments further support the view that bulk rhodopsin from disk and plasma membrane is similar, if not identical.

Recently, Boesze-Battaglia and Albert [33] have reported that photobleaching of plasma membrane rhodopsin in the presence of transducin, GTP and cGMP leads to low activation of cGMP-dependent phosphodiesterase relative to photobleaching of disk membrane rhodopsin. This effect has been attributed to the high concentration of cholesterol in the ROS plasma membrane which inhibits the Met I-Met II transition and transducin activation. In this study, we have investigated the light-induced phosphorylation of disk and plasma membrane rhodopsin by rhodopsin kinase. Results indicate that disk and plasma membrane rhodopsin are phosphorylated to the same extent. This suggests that phosphorylation of rhodopsin is not influenced by the lipid composition of the membrane.

In summary, the results reported here indicate that the bulk rhodopsin in the disk and plasma membrane are identical in respect to their structural properties, and ability to undergo light-induced, rhodopsin kinase catalyzed phosphorylation. This suggests that no differential modification occurs which may be responsible for the sorting of rhodopsin between disk and plasma membranes of rod outer segments during morphogenesis. However, one cannot rule out the possibility that a sorting signal is initially present during or after rhodopsin biosynthesis, but is removed when rhodopsin has reaches the outer segment. It is also possible that the sorting of rhodopsin between disk and plasma membrane is not a controlled process, but occurs randomly during ROS disk and plasma membrane morphogenesis. Other proteins such as the cGMP-gated channel [5], the Na^+/Ca^{2+}-K^+ exchanger [7], the GLUT-1 glucose transporter [9], peripherin/rds protein [11], and ROM-1 protein [12] which are preferentially sorted to either the plasma membrane or disk membrane, however, may be specifically sorted through membrane targeting sequences, post-translational modifications or interaction with cytoskeletal proteins.

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