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Phospholipase $C\gamma_1$ in Bovine Rod Outer Segments: Immunolocalization and Light-Dependent Binding to Membranes

*†‡Abboud J. Ghalayini, *Nathan R. Weber, §Dana R. Rundle, ||Cynthia A. Koutz, ||David Lambert, *†‡Xiao X. Guo, and *†‡§Robert E. Anderson

**Department of Ophthalmology, †Dean McGee Eye Institute, ‡Oklahoma Center for Neuroscience, and §Department of Biochemistry, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; and ||Department of Ophthalmology, Baylor College of Medicine, Houston, Texas, U.S.A.*

Abstract: We have investigated the isozymes of a phosphoinositide-specific phospholipase C (PLC) in bovine retina using several monoclonal antisera to PLCβ_1, γ_1, and δ_1. Immunoblot analysis showed that all three isozymes were present in the retina. Immunocytochemical localization in frozen bovine retina sections showed that PLCγ_1 was present in the photoreceptor cell layer, outer plexiform cell layer, inner plexiform cell layer, and ganglion cell layer. Immunoreaction within the photoreceptor cell layer was dependent on dark/light adaptation state of retinas. Immunoblot analysis of rod outer segments (ROS) with monoclonal or polyclonal antibodies to PLCγ_1 showed the presence of an immunoreactive band of 140 kDa. ROS prepared from retinas light-adapted in vitro had more PLCγ_1 on immunoblots than ROS from dark-adapted retinas. PLC enzyme activity in ROS from light-adapted retinas was 69 and 46% higher than ROS from dark-adapted retinas, when assayed in the presence and absence of ATP, respectively. This increase in enzyme activity was observed at $[Ca^{2+}]_{free}$ between 0.32 and 100 *µM.* These results demonstrate the presence of PLCγ_1 in bovine ROS and show that ROS prepared from light-adapted retinas are enriched in this isozyme, suggesting that light may promote the binding of this isozyme to bleached ROS membranes. **Key Words:** Phospholipase C—Rod outer segments—Immunocytochemistry—Phosphoinositides—Light.
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Bovine rod outer segments (ROS) contain a phosphoinositide-specific phospholipase C (PLC) activity. The specific identity of this enzyme is not known; however, in earlier studies, using a peptide-specific antiserum to the Y region of several PLCs, we have shown the presence of an ~140-kDa PLC in isolated ROS and the photoreceptor cell layer of frozen retina sections (Ghalayini et al., 1991). In other studies, PLCβ_4 has been cloned from a bovine retina library (Ferreira et al., 1993; Lee et al., 1993) and a human retina library (Alvarez et al., 1995). It has been immunolocalized to cone (Ferreira and Pak, 1994) and, more recently, to rod (Peng et al., 1997) photoreceptors. Interest in the identity of photoreceptor PLC is based on several lines of evidence indicating that this enzyme is light-activated (Ghalayini and Anderson, 1984; Hayashi and Amakawa, 1985; Millar et al., 1988; Pfeilschifter et al., 1988). Other reports have shown that PLC enzyme activity in ROS is regulated by arrestin (Ghalayini and Anderson, 1992) and calmodulin inhibitors (Ghem et al., 1991). In the current study, we have immunolocalized PLCγ_1 to bovine rod photoreceptor cells. We further demonstrate that ROS prepared from retinas bleached in vitro contain higher levels of PLCγ_1 and higher enzymatic activity than ROS prepared from dark-adapted retinas (DROS).

MATERIALS AND METHODS

Materials

$[2\text{-}^3H]$Inositol-labeled phosphatidylinositol 4,5-bisphosphate (PIP_2) was purchased from Du Pont-New England Nuclear (Boston, MA, U.S.A.). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) reagents, nitrocellulose sheets, and alkaline phosphatase-conjugated goat anti-rabbit and goat anti-mouse IgG were from Bio-Rad (Richmond, CA, U.S.A.). Dark-adapted frozen bovine retinas were from Excel (St. Louis, MO, U.S.A.). PIP_2, ATP, and other reagents were from Sigma (St. Louis).

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Address correspondence and reprint requests to Dr. A. J. Ghalayini at Department of Ophthalmology, Dean McGee Eye Institute, University of Oklahoma, 608 Stanton L. Young Boulevard, Room 409, Oklahoma City, OK 73104, U.S.A.

Abbreviations used: DROS, rod outer segments prepared from dark-adapted retinas; LROS, rod outer segments prepared from light-adapted retinas; Mab, monoclonal antibody; Pab, polyclonal antibody; PAGE, polyacrylamide gel electrophoresis; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; ROS, rod outer segments; SDS, sodium dodecyl sulfate.

Antisera

Monoclonal antibodies (Mabs) for PLCγ_1, PLCβ_1, and PLCδ_1 were obtained from Upstate Biotechnology (Lake Placid, NY, U.S.A.) and were used at a final concentration of 1 μg/ml. Polyclonal antibody (Pab; C-terminus-specific; catalogue no. sc-81) to PLCγ_1 was from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.) and was used at a concentration of 1 μg/ml. Polyclonal antisera to C-termini of PLCβ_1, PLCβ_2, and PLCβ_3 were a gift from Dr. Paul Sternweis and were used at a dilution of 1:400. Polyclonal antiserum 1109 [against a conserved sequence in the Y region of several PLC isozymes (Ghalayini et al., 1991)] was used at a dilution of 1:500 and was a gift from Dr. Alan Tarver. Polyclonal antiserum to arrestin was used at a dilution of 1:1,000 and was a gift from Dr. Igal Gery.

ROS preparation

Bovine DROS or ROS prepared from light-adapted retinas (LROS) were separated on a continuous sucrose gradient (25–50% wt/vol) as previously described (Ghalayini et al., 1991). LROS were prepared by exposing 50 dark-adapted bovine retinas for 20 min to room light (15–20 lux) in 50 ml of ice-cold buffer containing 10 m*M* Tris-HCl (pH 7.4), 100 m*M* NaCl, 2 m*M* $MgCl_2$, 20% sucrose, and 1 m*M* phenylmethylsulfonyl fluoride. DROS were prepared under dim red light, whereas LROS were prepared in room light. Whole-retina soluble fraction was obtained during ROS preparation as a supernatant from a 17,000-*g* (30-min) centrifugation of crude ROS membranes (Ghalayini et al., 1991). Hypotonic ROS extracts were obtained by suspending purified ROS (1–2 mg of protein) in 1.0 ml of 10 m*M* Tris-HCl (pH 7.5), followed by centrifugation at 100,000 *g* for 1 h.

SDS-PAGE and immunoblot analysis

SDS-PAGE was performed using 7.5% gels according to the procedure of Laemmli (1970). Resolved proteins were transferred to plastic-backed nitrocellulose sheets (pore size, 0.2 μm) using a Genie electroblotter (Idea Scientific Company, Minneapolis, MN, U.S.A.) for 2–3 h. Nitrocellulose sheets were blocked overnight at room temperature with Tris-buffered saline (pH 7.5) containing 0.1% Tween-20 and 5% crystalline-grade bovine serum albumin. Incubations with primary antisera (diluted in blocking buffer) were performed for 2–3 h (polyclonal antisera) or overnight (monoclonal antisera) at room temperature. Immunoreactions were detected with alkaline phosphatase conjugated to either goat anti-rabbit IgG or goat anti-mouse IgG, followed by alkaline phosphatase substrates nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate, *p*-toluidine salt. Densitometric scans of gels and immunoblots were analyzed by the ONE-DSCAN software program from Scanalytics (Billerica, MA, U.S.A.).

PLC enzyme assay

PLC activity in ROS was assayed as previously described (Ghalayini and Anderson, 1992) using exogenously added [^{3}H]PIP_2 as substrate. [^{3}H]PIP_2/phosphatidylethanolamine vesicles in a molar ratio of 1:1 were prepared by drying a mixture of the lipids under a stream of N_2 followed by sonication in 10 m*M* Tris-HCl buffer (pH 7.4), for 5 min in a bath sonicator. Incubations were conducted at 37°C for 20 min in 50 m*M* Tris-HCl buffer (pH 7.4), containing 1 m*M* EGTA, 1 m*M* $CaCl_2$ ($[Ca^{2+}]_{free}$ = 10 μM), 2 m*M* Mg^{2+}, 0.2% octylglycoside, 10 μM [^{3}H]PIP_2 (8,000–10,000 dpm), and 10 μg of ROS protein in a final volume of 100 μl. Calcium was buffered with 1 m*M* EGTA to give calculated $[Ca^{2+}]_{free}$ of 0.32, 0.72, 10, or 100 μM. Incubations were terminated by addition of 1.0 ml of chloroform/methanol (2:1 vol/vol), followed by addition of 0.1 ml of 1*M* HCl. The biphasic mixture was separated by centrifugation at 1,000 *g* for 5 min, and an aliquot (0.4 ml) of the upper phase was taken for scintillation counting.

Immunoprecipitation of PLCγ_1

LROS (3 mg of protein) in 10 m*M* Tris-HCl (pH 7.4), 100 m*M* NaCl, 2 m*M* $MgCl_2$, 10% sucrose, and 1 m*M* orthovanadate were spun at 40,000 *g* for 1 h, and the supernatant was removed and saved. The membranous pellet was resuspended in 0.3 ml of 5 m*M* Tris-HCl (pH 7.4) containing 1 m*M* $MgCl_2$ and spun at 40,000 *g* for 1 h. The two supernatants were pooled and adjusted to a final concentration of 150 m*M* NaCl and 50 m*M* Tris-HCl (pH 7.4) in a total volume of 1 ml. The pooled supernatants were precleared by addition of 50 μl of protein A-Sepharose for 3 h with gentle mixing at 4°C. The precleared supernatant was incubated with 5 μg of Pab PLCγ_1 and 50 μl of protein A-Sepharose at 4°C overnight with gentle mixing. The mixture was pulse-spun (15 s at 2,000 rpm in a microcentrifuge), and the pelleted beads were washed three times with 1.0 ml of buffer containing 50 m*M* Tris-HCl (pH 7.4), 150 m*M* NaCl, and 0.1% Triton X-100, followed by two washes with the same buffer without Triton X-100. The final beads were aliquoted and resuspended in either PLC assay buffer or SDS-PAGE sample buffer. Alternatively, PLCγ_1 was immunoprecipitated from ROS membranes, washed as described above, and solubilized in 50 m*M* HEPES (pH 7.5) containing 150 m*M* NaCl, 10% glycerol, 1% Triton X-100, 1.5 m*M* $MgCl_2$, 1 m*M* EDTA, and 1 m*M* orthovanadate. Detergent-insoluble material was removed by centrifugation at 40,000 *g* for 1 h. PLCγ_1 was immunoprecipitated from solubilized membranes as described above for the ROS supernatants.

Immunocytochemistry

Fresh bovine eyes obtained from a local abattoir were dark-adapted at 4°C for 2 h or light-adapted for 30 min under room light following the 2-h dark adaptation. Corneas were removed, and the eyecups were immersed in 4% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.3) for 4 days at 4°C. The eyecups were washed, further dissected into retinal-scleral pieces, and infiltrated for ~6 h in 5% sucrose in 0.1 *M* phosphate buffer (pH 7.3), followed by 30% sucrose in 0.1 *M* phosphate buffer (pH 7.3) overnight at 4°C. The tissue pieces were then frozen and sectioned at 5–10 μm on a Leica Cryocut 1800. The sections were hydrated, quenched with H_2O_2 (0.3%), rinsed with phosphate-buffered saline [10 m*M* Na_2HPO_4 (pH 7.4) and 100 m*M* NaCl] containing 0.25% Triton X-100, and incubated with primary antisera containing 0.25% bovine serum albumin and 0.25% Triton X-100 for 2 h at room temperature followed by 48 h at 4°C. Alternatively, incubations with polyclonal anti-PLCγ_1 were blocked with phosphate-buffered saline (pH 7.4) containing 0.25% Triton X-100, 0.25% bovine serum albumin, and 0.1% SDS for 2 h, followed by incubation with primary antisera for 2 h. In control incubations, polyclonal anti-PLCγ_1 (5 μg/ml) was neutralized by preincubation for 2 h at room temperature with 100 μg of C-terminus peptide corresponding to bovine brain PLCγ_1 amino acids 1,249–1,262. Sections were then rinsed with phosphate-buffered

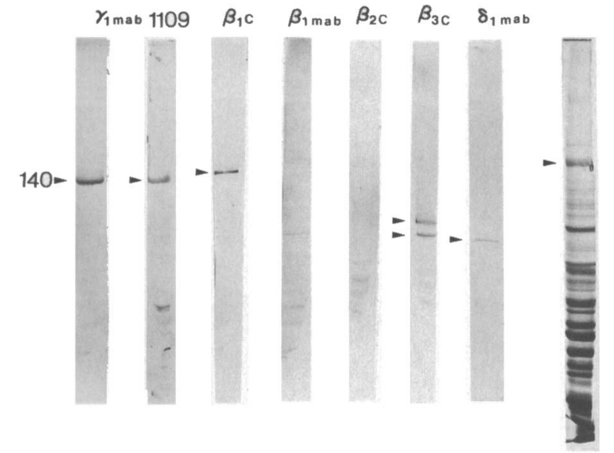

FIG. 1. Immunoblots of whole-retina soluble protein (67 μg) with anti-PLC antisera: Mab γ_1, Mab β_1, Ab 1109 (Y region), β_1 C-terminal, β_2 C-terminal, β_3 C-terminal, and Mab δ_1. Dilutions and other details are given in Materials and Methods.

saline, incubated for 1 h with biotinylated horse anti-mouse IgG or goat anti-rabbit IgG, rinsed, and incubated for 1 h with avidin–biotin complex (Vectastain kit; Vector, Burlingame, CA, U.S.A.). After rinsing, the peroxidase reaction was developed with 0.06% diaminobenzidine and 0.01% H_2O_2 in 0.05 *M* Tris-HCl buffer (pH 7.6) for 7–20 min at room temperature. Slides were then rinsed in tap water, coverslipped, and viewed on a Zeiss Axiovert Photomicroscope.

RESULTS

Identification of PLC isozymes in bovine retina and ROS membranes

Immunoblot analyses of whole-retina soluble proteins with Mabs to $PLC\gamma_1$, $PLC\beta_1$, and $PLC\delta_1$, Pabs to C-termini of $PLC\beta_1$, $PLC\beta_2$, and $PLC\beta_3$, and a Y region-specific Pab are shown in Fig. 1. $PLC\gamma_1$, $PLC\beta_1$, and $PLC\delta_1$, were all detected in the whole-retina soluble fraction (Fig. 1); however, only $PLC\gamma_1$ was detected in ROS membranes and ROS hypotonic extracts (Fig. 2, lanes 1 and 2, respectively). The presence of this isozyme in hypotonic extracts and washed ROS membranes suggests that it is either soluble or associated peripherally with ROS membranes.

Immunocytochemical localization of $PLC\gamma_1$ in bovine retina

Frozen sections from dark-adapted bovine retina incubated with Mab to $PLC\gamma_1$ are shown in Fig. 3. This antibody immunoreacted strongly with the photoreceptor cell layer and to a lesser extent with the outer plexiform cell layer. This reaction was specific, as control sections incubated with bovine serum albumin (C) or normal horse serum (CH) did not show any reaction. The immunoreaction within the photoreceptor cell layer appeared to be more pronounced in the inner segments. When sections from dark-adapted or light-adapted bovine eyes were probed with a Pab to $PLC\gamma_1$, immunoreaction in dark-adapted sections was similar to that observed with Mab to $PLC\gamma_1$ and was mainly in the photoreceptor inner segments. However, immunoreaction in sections from light-adapted retinas was dispersed throughout the photoreceptor cell layer (Fig. 4). Furthermore, immunoreaction in the photoreceptor cell layer, outer plexiform cell layer, and ganglion cell layers was abolished by preincubating the primary antibody with the C-terminus peptide (Fig. 4N) before addition to the sections, thus validating the specificity of the antibody reaction. Figure 4A shows that both antibodies (Mab and Pab $PLC\gamma_1$) used in immunocytochemistry recognize the same antigen of apparent molecular mass of 140 kDa.

Effect of light adaptation of bovine retina in vitro on PLC enzyme activity in isolated ROS membranes

DROS and LROS were assayed in the light for PLC enzyme activity in the presence and absence of 1 m*M* ATP (Fig. 5). PLC-specific enzyme activity in LROS was 46% higher than that of DROS when assayed in the absence of ATP. When enzyme activity was assayed in the presence of 1 m*M* ATP, PLC activity was significantly stimulated in both DROS and LROS; however, PLC enzyme activity in LROS was 69% higher than that of DROS. When PLC enzyme activity was assayed with various $[Ca^{2+}]_{free}$, PLC enzyme activity was higher in LROS than DROS at $[Ca^{2+}]_{free}$ between 0.72 and 100 μM (Fig. 6). In the presence of 1 m*M* ATP, the increase in PLC enzyme activity in LROS over DROS was most pronounced at $[Ca^{2+}]_{free}$ of 0.32–100 μM.

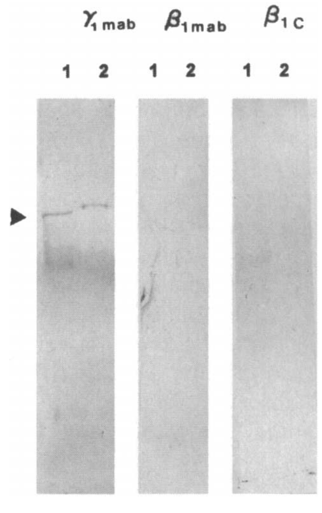

FIG. 2. Immunoblots of hypotonically washed ROS membranes (lane 1, 143 μg per lane) or ROS hypotonic extracts (lane 2, 13.7 μg per lane) with anti-PLC antisera: Mab γ_1, Mab β_1, and β_1 C-terminal. Dilutions and other details are given in Materials and Methods.

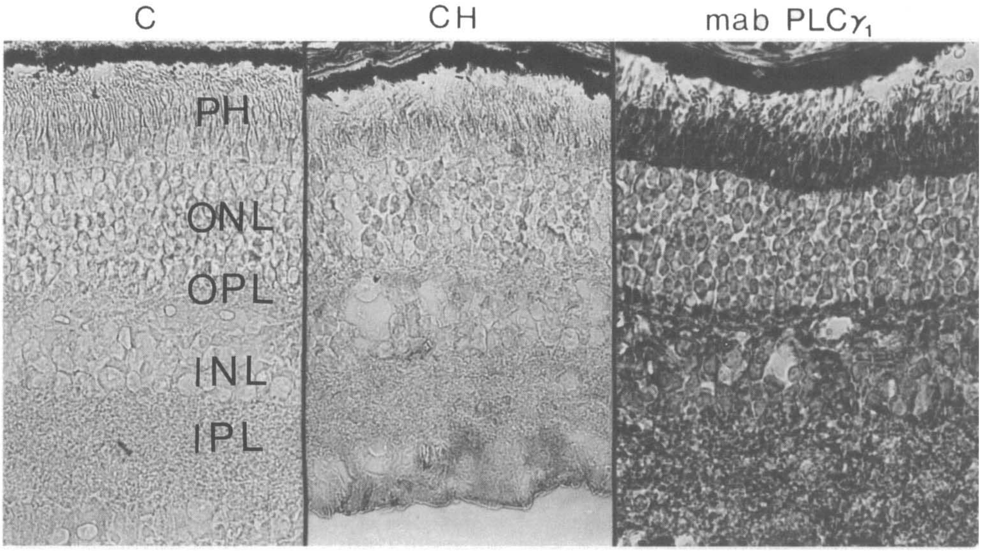

FIG. 3. Mab PLCγ_1: Immunocytochemistry of frozen sections from dark-adapted bovine retina (6–12 μm) with Mab to PLCγ_1. **C:** Control sections incubated with 0.3% bovine serum albumin. **CH:** Control sections incubated with normal horse serum. PH, photoreceptor cell layer; ONL, outer nuclear layer; OPL, outer plexiform cell layer; and INL and IPL, inner nuclear and plexiform layers, respectively.

Enrichment of LROS with PLCγ_1

Protein composition of ROS prepared from dark and light-adapted retinas is shown in Fig. 7B. LROS and DROS showed a similar polypeptide profile on Coomassie Blue-stained gels, with opsin being the predominant protein in both preparations. LROS appear to be enriched in at least three proteins of apparent molecular masses of ~140, 48, and 39 kDa over DROS. Immunoblot with anti-arrestin of DROS and LROS shows that the 48-kDa protein (indicated by arrows) is arrestin (Fig. 7C), whereas the 39-kDa protein is most likely to be the α-subunit of transducin, although its identity has not been determined. The identity of the 140-kDa protein is also uncertain; however, it is of a similar apparent molecular mass as PLCγ_1 (Fig. 1). When equal amounts of protein of DROS and LROS were subjected to immunoblot analysis with Mab PLCγ_1, the identity of the 140-kDa protein was shown to be PLCγ_1 (Fig. 7A). Densitometric quantification of PLCγ_1 from the immunoblot showed a 1.8-fold increase in the amount of this enzyme in LROS over DROS. Densitometric scans of the gel show that at least three additional proteins were enriched in LROS over DROS (Fig. 7D). Band 2 (arrestin) was enriched 1.7-fold, whereas bands 3 and 4 were enriched 1.2- and 1.3-fold, respectively. This enrichment of PLCγ_1 in LROS was observed in at least three independent preparations (three DROS and three LROS).

Immunoprecipitation of PLCγ_1

To verify further the presence of PLCγ_1 in bovine ROS, the enzyme was immunoprecipitated from LROS using a Pab to the C-terminus of PLCγ_1. Immunoprecipitates from ROS soluble extracts (pooled isotonic and hypotonic washes) or solubilized ROS membranes were subjected to immunoblot analysis with Mab to PLCγ_1 (Fig. 8). As shown, the immunoprecipitates from either soluble ROS extracts (lane 2) or solubilized ROS membranes (lane 4) contained an antigen that was recognized by Mab to PLCγ_1. Furthermore, the immunoprecipitated enzyme was catalytically active with specific activities of 0.25 and 0.42 pmol of PIP_2 hydrolyzed/min for the enzyme from ROS soluble extract and ROS membranes, respectively.

DISCUSSION

Interest in PLC enzyme activity in vertebrate photoreceptors is based on several independent observations indicating that this enzyme activity is light-activated (Ghalayini and Anderson, 1984; Hayashi and Amakawa, 1985; Millar et al., 1988; Pfeilschifter et al., 1988). Neither the identity of this light-activated PLC nor the specific mechanism of its light activation is understood. In the current report, we have identified and immunolocalized PLCγ_1 in photoreceptors of bovine retina. Moreover, we have demonstrated that the light history of isolated retinas may regulate both the enzyme activity and amount of PLCγ_1 protein associated with isolated ROS membranes. Based on densitometric scans of immunoblots of LROS and DROS in vitro, LROS contained significantly more PLCγ_1 protein than DROS. This observation suggests that light adaptation of retinas in vitro may promote the binding of this isozyme to ROS membranes. In addition, PLC enzyme activity assayed in vitro was significantly higher in LROS than in DROS. Although the latter observation does not allow us to predict which PLC isozyme is responsible for the observed increase in enzyme activity, it is strongly suggestive that it may

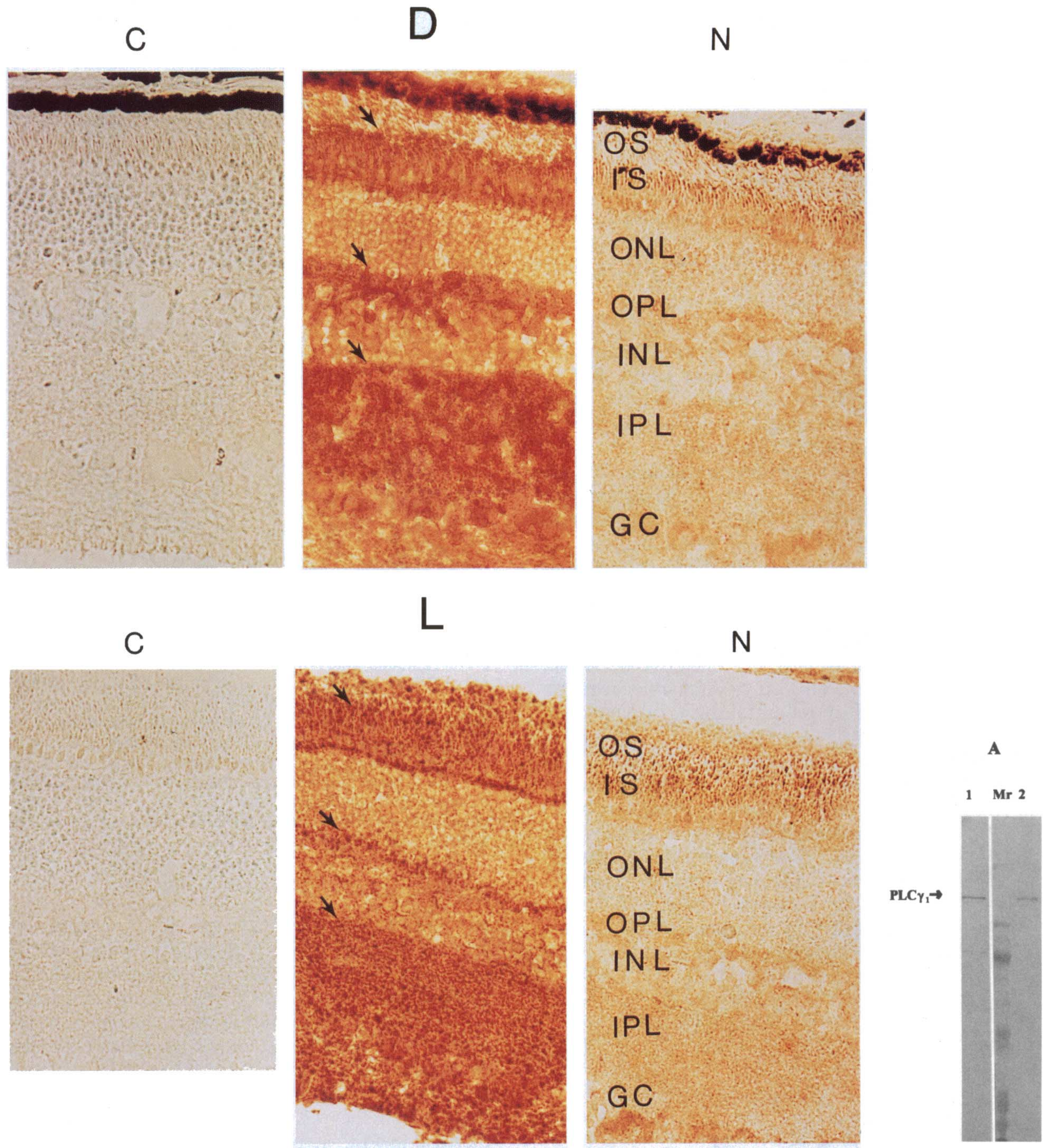

FIG. 4. Immunocytochemistry of bovine retina frozen sections (8 μm) from dark- (**D**) and light-adapted (**L**) eyes with C-terminus-specific anti-PLCγ_1 at a concentration of 5 μg/ml. **C:** Control sections incubated with 0.3% bovine serum albumin. **N:** Control sections incubated with neutralized anti-PLCγ_1 (preincubated with C-terminus peptide; see Materials and Methods for details). Immunoreaction is indicated by arrowheads. OS, photoreceptor outer segments; IS, photoreceptor inner segments; ONL, outer nuclear layer; OPL, outer plexiform cell layer; INL and IPL, inner nuclear and plexiform layers, respectively; and GC, ganglion cell layer. **A:** Immunoblot of bovine ROS membranes (100 μg of protein) with either Pab to PLCγ_1 (lane 1) or Mab to PLCγ_1 (lane 2) at a concentration of 1 μg/ml each.

be due to increased levels of PLCγ_1 in LROS. To date several isozymes of PLC, including PLCγ_1, PLCβ_1, and PLCδ_1 (this study) and PLCβ_1, PLCβ_3, PLCβ_4, PLCγ_1, PLCδ_1, and PLCδ_2 (Lee et al., 1993), have been identified in retina. In one report, PLCβ_4 was immunolocalized to cones (Ferreira and Pak, 1994).

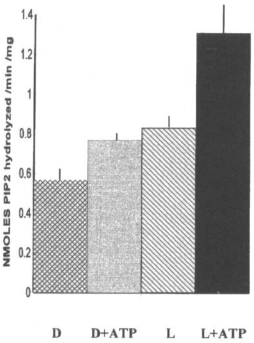

FIG. 5. PLC enzyme activity was assayed as described in Materials and Methods. D, dark-adapted; L, light-adapted. Assay mixtures contained 10 μg of ROS protein incubated for 20 min at 37°C at 10 μM $[Ca^{2+}]_{free}$, in the presence and absence of 1 mM ATP. Data are mean ± SD (bars) values from two experiments performed in duplicate.

More recently this isozyme was immunolocalized to ROS (Peng et al., 1997). In the latter report, PLCγ_1 was localized to the outer plexiform cell layer, inner plexiform cell layer, and ganglion cell layer. However, it was not detected in the photoreceptor cell layer, which is in conflict with our current findings. The apparent discrepancy in the cellular localization of PLCγ_1 may be due to (a) differences in specificity of antisera used, (b) difference in tissue fixation and preparation, or (c) differences in the dark and light adaptation states of the retina. As we show in this report, light adaptation results in a significant difference in the immunoreaction within photoreceptors. In more recent studies, we have observed (Weber et al., 1997) a similar effect on PLCγ_1 immunolocalization in photoreceptors of rat retina following in vivo light exposure.

Of all the known PLC isozymes, PLCγ (γ_1 and γ_2) are the only isozymes that have been shown in nonocular tissues to be activated by phosphorylation by either receptor or nonreceptor tyrosine kinases (for reviews, see Rhee, 1992; Schlessinger, 1997). The observed specific effect of ATP and not GTP (data not shown) on PLC activity in this study may be indicative of phosphorylation of PLCγ_1 or, alternatively, suggest the phosphorylation of another protein that might regulate PLC activity. We have previously reported that arrestin activates ROS PLC in vitro (Ghalayini and Anderson, 1992); however, in these earlier studies, the specific identity of the PLC activated by arrestin was not determined. In the current study, the increased level of PLCγ_1 in LROS is accompanied by an enrichment of arrestin in these membranes (Fig. 7C), which has been shown to bind to bleached and phosphorylated ROS membranes (Kühn, 1984). Thus, the observed increase in PLC activity in LROS may be due to the enrichment of PLCγ_1, arrestin, or both proteins in these membranes. Based on the current observation and the earlier effects of arrestin on ROS PLC activity, we propose that PLCγ_1 is a likely candidate for activation by arrestin in ROS. Several recent reports have shown that PLCγ_1 associates with cytoskeletal proteins and binds to these proteins in response to the appropriate stimulus (Seedorf et al., 1994; Yang et al., 1994). Similarly, light might stimulate PLCγ_1 by promoting its binding to bleached ROS membranes. Earlier studies on the effect of light on PLC activity (as-

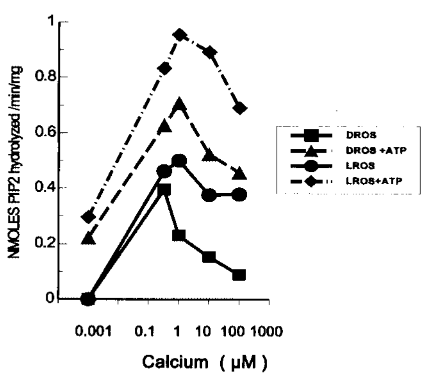

FIG. 6. Effect of $[Ca^{2+}]_{free}$ on PLC enzyme activity from DROS or LROS in the presence and absence of 1 mM ATP. Data were obtained from a single experiment and are representative of two other experiments.

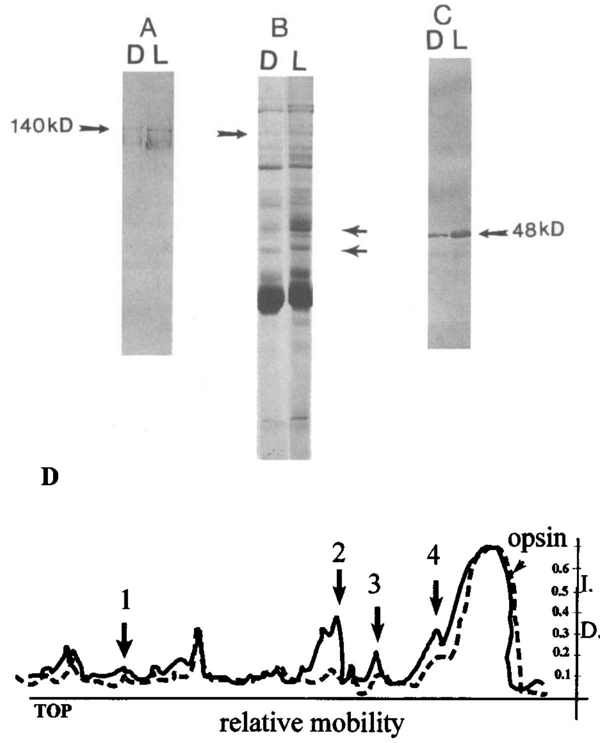

FIG. 7. A: Immunoblot of DROS (D) and LROS (L; 100 μg of protein per lane) with Mab to PLCγ_1. **B:** Coomassie Blue-stained gel of DROS and LROS (50 μg of protein per lane). **C:** Immunoblot of DROS and LROS (100 μg of protein per lane) with anti-arrestin antibody (1:1,000 dilution). **D:** Densitometric scan of Coomassie Blue-stained gel (integrated density; I. D., arbitrary units) for LROS (solid trace) and DROS (dashed trace). Band 1, PLCγ_1; band 2, arrestin; band 3, 39-kDa protein; and band 4, 37-kDa protein. Quantification of PLCγ_1 from blot and other protein bands from the Coomassie Blue-stained gel was analyzed by ONE-DSCAN software.

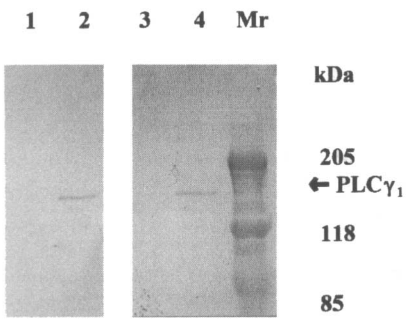

FIG. 8. Immunoprecipitation of $PLC\gamma_1$ from solubilized ROS membranes with Pab to $PLC\gamma_1$ was performed as described in Materials and Methods. Immunoprecipitates from pooled soluble extracts of ROS membranes (lane 2) or solubilized ROS membranes (lane 4) were subjected to immunoblot analysis with Mab to $PLC\gamma_1$ at a concentration of 1 μg/ml. Lanes 1 and 3 are corresponding immunoprecipitates obtained by incubation with protein A-Sepharose without Pab to $PLC\gamma_1$. Mr, molecular mass standards.

sayed with exogenous substrate in vitro) in ROS membranes isolated from dark-adapted retinas have been inconclusive (A.J.G. and R.E.A., unpublished data). Light exposure in the current study was performed in vitro on isolated retinas before preparation of ROS. Thus, the observed difference in PLC activity between LROS and DROS may require an intact photoreceptor or retina to occur. As such, the observed increase in PLC activity in LROS may represent an adaptive light response that is retained biochemically in isolated ROS. In an earlier report, we have observed that similarly prepared LROS incorporated three- to fivefold more *myo*-[^{3}H]inositol into phosphoinositides than DROS (Ghalayini and Anderson, 1995), which indicates that phosphatidylinositol synthetase may also be regulated by light. More recently, we have observed that phosphatidylinositol 3-kinase enzyme activity was also increased in LROS (Guo et al., 1997). The cumulative data strongly suggest that several phosphoinositide-metabolizing enzymes may be regulated by light. Based on the current and previous observations, we propose that light exposure in vitro of intact retina may provide a useful model for specifically studying the light-activated phosphoinositide cycle in photoreceptors.

The functional significance of the light-activated PLC in photoreceptors is not clear. However, downstream signals originating with the activation of PLC appear to play a role in photoreceptor desensitization/adaptation. Specifically, protein kinase C has been shown to phosphorylate both bleached rhodopsin (Newton and Williams, 1991; Udovichenko et al., 1997) and the inhibitory subunit of cyclic GMP phosphodiesterase (Udovichenko et al., 1993, 1994). Phosphorylation of rhodopsin and the cyclic GMP phosphodiesterase γ subunit may contribute to the desensitization of the visual cycle. Protein kinase $C\alpha$ has been detected (Udovichenko et al., 1993) and purified (Wolbring and Cook, 1991) from ROS membranes. In another study, Williams et al. (1993) reported the presence of a novel Ca^{2+}- and diacylglycerol-sensitive protein kinase C. These protein kinase C isozymes require both calcium and diacylglycerol for their activity. Diacylglycerol generated from hydrolysis of PIP_2 by a light-activated PLC could trigger one or both of these adaptive events. Recent studies in nonocular tissue have shown that PIP_2 may play the role of second messenger by (a) regulating membrane ion channels/exchangers (Hilgemann and Ball, 1996), (b) serving as a membrane docking site for proteins containing pleckstrin homology domains (Lemmon et al., 1995; Kubiseski et al., 1997), (c) regulating nucleotide exchange (Zheng et al., 1996), (d) regulating GTPase activity (Lin and Gilman, 1996), (e) activating G protein-coupled receptor kinases (Pitcher et al., 1996), or (f) regulating vesicular trafficking and cytoskeletal organization (De Camilli et al., 1996). Several of these cellular events are likely to occur within photoreceptor cells. Thus, light-mediated decreases in PIP_2 could play a similar regulatory role in events that are important to either the structural or functional integrity of photoreceptor cells.

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