The Effect of Lipid Environment and Retinoids on the ATPase Activity of ABCR, the Photoreceptor ABC Transporter Responsible for Stargardt Macular Dystrophy*

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ABCR is a photoreceptor-specific ATP-binding cassette transporter that has been linked to various retinal diseases, including Stargardt macular dystrophy, and implicated in retinal transport across rod outer segment (ROS) membranes. We have examined the ATPase and GTPase activity of detergent-solubilized and reconstituted ABCR. 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonic acid-solubilized ABCR had ATPase and GTPase activity ($K_m$ = 75 μM, $V_{max}$ = 200 nmol/min/mg) that was stimulated 1.5-2-fold by all-trans-retinal and dependent on phospholipid and dithiothreitol. The $V_{max}$ for ATP decreased to $\sim 25$ μM after reconstitution, whereas the $V_{max}$ was strongly dependent on the lipid used for reconstitution. ABCR reconstituted in ROS phospholipid had a $V_{max}$ for basal and retinal activated ATPase activity that was 4-6 times higher than for ABCR in soybean or brain phospholipid. This enhanced activity was mainly due to the high phosphatidylethanolamine (PE) content of ROS membranes. PE was also required for retinoid-stimulated ATPase activity. ATPase activity of ABCR was stimulated by the addition of N-retinylidene-PE but not the reduced derivative, retinyl-PE. ABCR expressed in COS-1 cells also exhibited retinal-stimulated ATPase activity similar to that of the native protein. These results support the view that ABCR is an active retinoid transporter, the nucleotidase activity of which is strongly influenced by its lipid environment.

ABCR, also known as the rim protein, is an abundant high molecular weight membrane glycoprotein found in photoreceptor outer segment disc membranes (1-3). Primary structural analysis indicates that ABCR is a member of the superfamily of ATP-binding cassette proteins that typically function in the active transport of various substances across cell membranes (2, 4, 5). Like other eukaryotic ABC transporters, such as P-glycoprotein and cystic fibrosis transmembrane conductance regulator, ABCR is organized in two homologous, tandem-arranged halves, each containing a cytoplasmic nucleotide binding domain preceded by a hydrophobic domain consisting of multiple membrane spanning segments.

The gene encoding ABCR has been implicated in a variety of retinal degenerative diseases associated with a loss in vision. Over 80 different mutations in ABCR have been found in patients with Stargardt macular dystrophy, a juvenile onset, autosomal recessive disease characterized by decreased visual acuity, bilateral atrophy of the central (macula) retina, accumulation of fluorescent yellow deposits in the retinal pigment epithelium, and delayed dark adaptation (4, 6-8). Mutations in ABCR have also been linked to individuals with fundus flavimaculatus, a late-onset variant of Stargardt macular dystrophy (9), autosomal recessive retinitis pigmentosa (10), cone-rod dystrophy (11), and age-related macular dystrophy (12, 13).

The substrate(s) transported by ABCR is not yet known. However, localization of ABCR to photoreceptor outer segment disc membranes led to the initial suggestion that ABCR may function to transport retinoids across the disc membrane (2-4). The putative role of ABCR as a retinal transporter is supported by two recent studies. In one study, purified ABCR reconstituted into brain lipid vesicles displayed ATPase activity that was stimulated up to 5-fold by retinal (14). Substrates that are actively transported across cell membranes by P-glycoprotein, histidine permease, multidrug resistance-associated protein, and the canalicular multispecific organic anion transporter (MOAT/multidrug resistance-associated protein 2) also activate the ATPase activity of these proteins (15-22). In a second study, an $\alpha$-knockout mouse has been produced that displays delayed dark adaptation, a light-dependent increase in all-trans-retinal and protonated N-retinylidene-PE in ROS, and an accumulation of the pyridinium bis-retinoid compound, A2E, in photoreceptors and retinal pigment epithelial cells (23). Many of these characteristics are observed in individuals with Stargardt disease and are consistent with an accumulation of retinal-PE derivatives in photoreceptor membranes presumably due to defective transport of retinoid compounds across disc membranes.

As part of an ongoing study to characterize the structural and functional properties of ABCR, we have investigated the effect of various phospholipids and retinoid compounds on the nucleotidase activity of ABCR from ROS membranes. Here, we report that detergent-solubilized and reconstituted ABCR displays both ATPase and GTPase activity that is strongly influenced by the lipid environment and the presence of retinoid...
compounds. We also show that ABCR expressed in monkey kidney COS-1 cells exhibits retinal-stimulated ATPase activity comparable to that of the native protein.

**EXPERIMENTAL PROCEDURES**

**Reagents**—All-trans-retinal, all-trans-retinol, reduced glutathione, soybean phospholipid, SDPC, CHAPS, and N-ethylmaleimide were purchased from Sigma. Citral, 2,4-all-trans-nonaenal, 2-trans-6-cis-nonaenal, and nonylaldehyde were from Aldrich. 11-cis-Retinal was a generous gift of Dr. Rosalie Crouch. The following phospholipids were obtained from Avanti Polar Lipids: SDPE, DOPE, DOPC, and brain polar lipid extract.

**Solutions**—The composition of buffers was as follows: homogenization buffer, 20 mM Tris acetate, pH 7.4, 10 mM taurine, 10 mM glucose, 0.25 mM MgCl₂, 20% (w/v) sucrose; column buffer (Buffer C), 50 mM HEPES, pH 7.5, 0.1 M NaCl, 10 mM CHAPS, 1 mg/ml sonicated soybean phospholipid, 1 mM DTT, 3 mM MgCl₂, 10% (v/v) glycerol; Buffer E, 50 mM HEPES, pH 7.5, 0.1 mM NaCl, 0.5 mM EDTA, 10 mM CHAPS, 1 mM DTT, 10% glycerol; dialysis buffer (Buffer D1), 10 mM HEPES, pH 7.5, 0.1 mM NaCl, 1 mM DTT; Buffer D2, 50 mM HEPES, pH 7.5, 0.1 mM NaCl, 1 mM DTT, 3 mM MgCl₂, 10% glycerol; and reconstitution buffer (Buffer E), 25 mM HEPES, pH 7.5, 140 mM NaCl, 1 mM EDTA, 1 mM DTT, 10% glycerol. Retinoid compounds were dissolved in ethanol and diluted at least 100-fold in Buffer C or Buffer E (final ethanol concentration, <0.5% in reaction). All-trans-retinal, 11-cis-retinal, and all-trans-retinol concentrations were determined spectrophotometrically in ethanol using molar extinction coefficients of 42,880 ($\lambda_{max}$ = 385 nm), 24,935 ($\lambda_{max}$ = 380 nm), and 52,770 ($\lambda_{max}$ = 325 nm), respectively (24).

**Preparation and Purification of All-trans-Retinal-PE Conjugates—**N-Retinylidene-PE, the Schiff base conjugate of retinal and PE, was prepared by the method of Anderson and Maude (25). All-trans-retinal (2.0 μmol) was mixed with 2.0 μmol of DOPE in 1.0 ml of a solvent consisting of chloroform, methanol, and triethylamine (12:6:1, by volume). The reaction was shielded from light and allowed to proceed for at least 30 min at room temperature. For the preparation of the reduction product of N-retinylidene-PE, N-retinyl-PE, a 1000-fold molar excess of NaBH₄ was added following the initial incubation period. The retinal-PE conjugates were purified by HPLC on a Phenomenex Primesphere 5 C18 HC column (150 × 3.2 mm) by a procedure adapted from Parish et al. (26). The samples were eluted using a continuous gradient of 85% methanol in water to 100% methanol over a period of 30 min, followed by isocratic elution with 100% methanol (all solvents also contained 0.1% trifluoroacetic acid), at a flow rate of 0.5 ml/min (Fig. 1). Retinylidene-PE was titrated at approximately 12 min, N-retinylidene-PE at 48 min, and N-retinyl-PE at 60 min. N-Retinylidene-PE eluted from the column as the protonated Schiff base with an absorption maximum of 450 nm. Upon deprotonation of the Schiff base by addition of 5 N NaOH, the absorption maximum shifted to 370 nm, consistent with earlier observations (25). The absorption maximum of the N-retinyl-PE peak was 329 nm. The mass of the N-retinyl-DOPE compound was verified by electrospray mass spectrometry.

**Isolation of Rod Outer Segments**—ROS membranes were isolated from previously frozen bovine retinas on a continuous sucrose density gradient as described previously (27) and stored in homogenization buffer (4–8 mg protein/ml) at –80 °C.

**Extraction of Phospholipids from ROS Membranes**—Phospholipids were extracted from ROS membranes by the method of Folch et al. (28), with precautions to prevent the reaction of endogenous retinal with amine-containing phospholipids and to limit the oxidation of the polyunsaturated acyl chains that are abundant in ROS phospholipids (29, 30). ROS membranes (16 mg) were washed three times in 10 mM potassium phosphate, pH 7.0, and suspended in 0.8 ml of the same buffer. A solution of 0.8 ml of 1 M NH₄OH (pH adjusted to 7.0 using NaHCO₃) and 4.2 ml of methanol was added to the membranes, and the mixture was incubated on ice for 10 min. NH₂OH derivatized retinal to the protonated Schiff base with an absorption maximum of 450 nm. The mixture was incubated on ice for 30 min. Buffer E (400 μl) was added, and the mixture was incubated on ice for 30 min. Buffer E (400 μl) was added, and the vesicles were passed through 400 μl of Extracti-gel resin in a 10 μm pore size filter Mobicol mini-column (MobiTec, Gottingen, Germany) that had been equilibrated in Buffer E. The flow-through containing reconstituted ABCR was collected at 0.8 ml/min by applying gentle pressure with a syringe, and 5 mM MgCl₂ was added prior to measuring ATPase activity.

**ATPase Assay—**The hydrolysis of [γ-³²P]ATP (NEN Life Science
ATPase Activity of ABCR

Products) in a 10-μl reaction volume was detected by thin layer chromatography as described before (32). Eight μl (20–40 ng) of CHAPS-solubilized ABCR (diluted in Buffer C) or reconstituted sample (undiluted) were pipetted into 0.5-ml microcentrifuge tubes. Retinal and other compounds were added from 10× solutions. The reaction was initiated with the addition of 1 μl of a 10× ATP solution (0.2 μCi). Unless otherwise noted, the final ATP concentration was 1 mM for solubilized ABCR and 50 μM for reconstituted ABCR. After 30 min at 37 °C, 4 μl of 10% SDS were added, and the tube was centrifuged briefly. One μl of the reaction mixture was spotted onto a polyethyleneimine cellulose plate (Aldrich) and chromatographed in 0.5 M LiCl/1 M formic acid. The plate was exposed to a storage phosphor screen for 3 h and scanned in a PhosphorImager SI (Molecular Dynamics, Sunnyvale, CA). Spots corresponding to ATP and ADP were quantified using IPLab Gel Analysis software (Signal Analytics Corp., Vienna, VA). The ratio of the amount of ADP produced to the initial amount of ATP present in the reaction mixture was calculated. Each sample was assayed in triplicate. Buffer blanks were included to determine nonenzymatic ATP hydrolysis, which was subtracted from the total. GT-Pase activity was measured in an identical manner using [γ-32P]GTP.

Protein Determination—The protein concentration of ROS membrane preparations was determined by the BCA method (Pierce). The amount of protein in detergent extracts was determined by comparing the intensity of Coomasie Brilliant Blue staining that with that of bovine serum albumin standards after SDS-polyacrylamide gel electrophoresis. Protein content of reconstituted ABCR was estimated by Western blot analysis using known amounts of purified ABCR protein. Gels and film were scanned with an Ultrascan XL densitometer (LKB, Bromma, Sweden), and relative peak areas were used to determine protein concentration.

Western Blot Analysis—Proteins were separated by SDS gel electrophoresis on 6% polyacrylamide gels and transferred to Immobilon P membranes (Millipore) at 300 mA for 40 min in a semidry transfer apparatus (Bio-Rad) using a buffer consisting of 25 mM Tris, 192 mM glycine, 10% methanol, pH 8.3. The membrane was incubated in 1% skim milk, PBS (140 mM NaCl, 3 mM KCl, 10 mM phosphate, pH 7.4) containing 0.05% Tween 20 (PBS-T) for 30 min and incubated first with Rim 3F4 monoclonal antibody diluted in 0.1% milk, PBS-T for 1 h and then with peroxidase-conjugated sheep anti-mouse IgG (diluted 1:5000 in 0.1% milk, PBS-T) for detection by enhanced chemiluminescence (Amersham Pharmacia Biotech).

COS-1 Cell Expression—For expression studies, the human ABCR cDNA (4, 6) was subcloned into the NorI and blunted XhoI restriction sites of pcDNA3 (Invitrogen). COS-1 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin and passaged twice a week. Cells (one 10-cm dish) were transfected at 80% confluency with 5 μg of pcDNA3-ABCR and 60 μl of SuperFect transfection reagent (Qiagen) for 3 h. After 48 h, cells were washed twice in PBS and harvested by scraping in PBS. The cells were centrifuged at 2800 × g for 10 min, and the pellet was solubilized in 0.5 μl of Buffer C containing 15 mM CHAPS and 0.2 mM phenylmethylsulfonyl fluoride. ABCR was purified on a Rim3F4-Sepharose 2B column as described above for ABCR from ROS membranes, except that the procedure was carried out under normal laboratory light. Typically, four 10-cm dishes of transfected cells were used for 100 μl of packed Rim3F4 beads.

RESULTS

Nucleotidase Activity of CHAPS-solubilized ABCR—The ATPase activity of CHAPS-solubilized, immunoaffinity-purified ABCR from ROS membranes was measured under various conditions. When the purification and activity measurements were carried out in the presence of soybean phospholipids, DTT, and glycerol, ABCR exhibited a basal ATPase activity that was stimulated up to 2-fold by 11-cis- and all-trans-retinal (Figs. 2 and 3A). All-trans-retinol, reduced glutathione, and four structurally related unsaturated aldehydes (citral, 2,4-nonenal, 2,6-nonenal, and nonyl aldehyde) had no significant effect on the basal ATPase activity of ABCR. The activity of CHAPS-solubilized ABCR was dependent on the presence of DTT and lipid. Over 70% of the basal ATPase activity was lost when DTT or soybean phospholipid was omitted during purification and activity determination. N-Ethylmaleimide also inhibited the ATPase activity of ABCR.

The ATPase activity of solubilized ABCR was highly labile. ATPase stimulation by all-trans-retinal was lost when CHAPS-solubilized ABCR was stored overnight at 4 °C. Preincubation of ABCR at 37 °C for 30 min resulted in the loss of over 90% of the ATPase activity of ABCR. Addition of ATP during preincubation diminished this inactivation. Approximately 40% of the basal ATPase activity remained if the preincubation step was carried out in the presence of 1 mM ATP.

CHAPS-solubilized ABCR exhibited both ATPase and GT-Pase activities. As shown in Fig. 3, the basal ATPase and GT-Pase activities were activated by all-trans-retinal with half-maximal stimulation at 10 μM all-trans-retinal. The specific ATPase and GT-Pase activities were similar.

ATPase Activity of ABCR Reconstituted into Phospholipid Vesicles—Previously, Sun et al. (14) reported that ABCR reconstituted into brain polar lipid and brain PE vesicles basal and retinal-stimulated ATPase activity. We have now reconstituted purified ABCR into various phospholipid mixtures in order to examine further the effect of the lipid environment on the basal and retinal-stimulated ATPase activity of ABCR. Fig. 4 shows the dependence of ATP hydrolysis on ATP concentration for ABCR reconstituted into ROS phospholipid vesicles. The $K_{\text{m}}$ and $V_{\text{max}}$ values for ATP hydrolysis by detergent-solubilized
and reconstituted ABCR are compared in Table I. The $K_m$ values for basal ATPase activity of ABCR reconstituted in soybean, brain, and ROS phospholipid are similar ($K_m \sim 25 \mu M$ ATP) but generally lower than for CHAPS-solubilized ABCR ($K_m \sim 75 \mu M$ ATP). The lipid composition had a significant influence on the $V_{max}$ values of the reconstituted enzyme. The $V_{max}$ of ABCR reconstituted into ROS phospholipid ($V_{max} = 202 \pm 45 \text{ nmol/min/mg}$) is four times higher than ABCR reconstituted into soybean phospholipid and over six times higher than ABCR in brain polar lipid but comparable to that observed for the solubilized protein (Table I). Retinal increased both the $V_{max}$ and $K_m$ for ATP hydrolysis by ABCR reconstituted into each type of lipid. Although retinal stimulation was somewhat variable between preparations, in general, retinal increased the $V_{max}$ by 2.5–5-fold for ABCR in ROS and brain lipids but only 1.5–2-fold for ABCR in soybean phospholipids.

In addition to affecting the kinetics of ATP hydrolysis, the lipid environment also influenced the stability of ABCR. Unlike CHAPS-solubilized ABCR, the basal and retinal-stimulated activity of reconstituted ABCR was unaffected by storage at $4 ^\circ C$ for at least 4 days.

The effect of all-trans-retinal and all-trans-retinol concentration on the ATPase activity of ABCR in ROS and brain phospholipids was also measured. Fig. 5 shows that ATPase activity reached a maximum at about 50 $\mu M$ all-trans-retinal. At high retinal concentration (200 $\mu M$), an inhibition of ATPase activity was typically observed for ABCR reconstituted in ROS lipids. All-trans-retinol also stimulated the ATPase activity of ABCR reconstituted in brain and ROS lipids, but this activation was less pronounced and occurred only at higher retinol concentration.

**Effect of Different Lipids on ATPase Activity**—ROS membranes are known to contain a relatively high content of PE and docosahexaenoic acid (C22:6) containing phospholipids (25, 30). To determine whether these lipids are responsible for the increased ATPase activity of ABCR in ROS lipids, we measured the basal and retinal-stimulated ATPase activity of ABCR reconstituted into brain lipid vesicles containing added SDPE, SDPC, DOPE, or DOPC (% of brain lipid extract replaced by pure PE or PC) and assayed for ATPase activity in the absence or presence of 50 $\mu M$ all-trans retinal. All lipids were dissolved in chloroform:methanol (1:1), mixed together, and dried under nitrogen before dissolving in buffer and bath sonication. The final lipid concentration was kept constant at 0.7 mg/ml. The means of three experiments ± S.D. are shown.

**Effect of N-Retinylidene-PE on ATPase Activity of ABCR**—It has long been known that retinal reacts with PE to form the Schiff base conjugate, N-retinylidene-PE (25, 33). To determine the extent to which retinal reacts with PE in lipid vesicle preparations used for reconstitution, 50 $\mu M$ all-trans-retinal was added to brain polar lipid vesicles (lipid concentration, 0.8 mg/ml) at 37 °C. After a 30-min incubation period, the reaction was stopped by the addition of NaBH$_4$, and the retinoids and N-retinyl-PE products were separated by HPLC (Fig. 7) and quantified from the peak absorbances and extinction coeffi-
cients of these compounds (25, 33). Approximately 55% (mole ratio) of the all-trans-retinal added to the vesicles reacted with PE to form N-retinylidene-PE derivatives.

Given that retinal can form a conjugate with PE in vesicles, it was of interest to determine whether exogenously added N-retinylidene-PE would have any effect on the ATPase activity of ABCR. As shown in Fig. 8, the addition of N-retinylidene-PE to ABCR reconstituted in brain polar lipid resulted in stimulation of the ATPase activity by approximately 3-fold. The addition of 1.0–25.0 μM N-retinylidene-PE (NaBH₄-reduced compound), instead of N-retinylidene-PE, resulted in a small inhibition in ATPase activity of about 25%.² There was no significant stimulation of ATPase activity by 25 μM N-retinylidene-PE, all-trans retinal, or PE when ABCR was reconstituted in pure SDPC or DOPC vesicles (Fig. 9).

The Schiff base formed between retinal and PE is known to be labile. Therefore, we investigated whether N-retinylidene-PE was stable when added to brain polar lipid vesicles or whether it dissociated into its parent compounds. To this end, N-retinylidene-PE at an initial concentration of 10 μM was incubated with vesicles for up to 30 min. Subsequent HPLC analysis revealed that approximately 40% of the N-retinylidene-PE was recovered as the retinal-PE conjugate, whereas the rest was recovered as the free retinoid.

Expression of the Human ABCR in COS-1 Cells—To determine whether the retinal-stimulated ATPase activity observed in purified ABCR preparations is a property of ABCR itself, rather than a contaminating ATPase of ROS membranes, ABCR was expressed in monkey kidney COS-1 cells, and the ATPase activity of the immunoaffinity-purified protein was measured in the presence and absence of retinal. Fig. 10 shows a Coomassie Blue-stained gel and a Western blot of ABCR purified from transfected COS-1 cells. As in the case of ABCR from ROS membranes, the expressed and purified ABCR migrated on SDS gels as a single protein with an apparent molecular mass of 220 kDa. Over 50% of ABCR expressed in COS-1 cells bound to the Rim 3F4 immunoaffinity matrix. After reconstitution into brain phospholipid vesicles, ABCR showed basal and retinal-stimulated ATPase activity similar to that of the native protein (Fig. 11).

² J. Ahn, J. T. Wong, and R. S. Molday, unpublished observations.

DISCUSSION

In this study, we have examined the effect of lipids and other compounds on the nucleotidase activity of ABCR purified from ROS disc membranes. Phospholipid and a reducing environment are required because ATPase activity is lost when deter-
experiments). A mock purification using untransfected COS-1 cells (UNTR) was also performed to measure background ATPase activity. ATP hydrolysis was measured in the absence (open columns) or presence of 50 μM all-trans-retinal (solid columns). The amount of ATP hydrolyzed over a 30-min period by immunopurified and reconstituted protein from approximately 2 × 10⁶ cells is shown ± S.D. (three experiments).

The lipid environment influences the kinetics of ATP hydrolysis by ABCR. A 3-fold decrease in the $K_m$ for basal ATPase activity occurs upon reconstitution of ABCR into lipid vesicles. Removal of detergent and/or the presence of a lipid bilayer may favor the formation of the enzyme-substrate complex between ATP and ABCR. The lipid environment also affects the $V_{max}$ for ATP hydrolysis. The $V_{max}$ values for ABCR reconstituted into soybean or brain phospholipid vesicles are considerably lower than for detergent-solubilized ABCR (Table I). The soybean and brain phospholipid bilayer may constrain conformational changes in ABCR coupled to ATP hydrolysis, thereby resulting in a decrease in reaction rate. Interestingly, the $V_{max}$ of ABCR reconstituted into ROS disc lipids is considerably higher than the $V_{max}$ of ABCR reconstituted into brain or soybean lipids and comparable to the basal activity of purified P-glycoprotein, cystic fibrosis transmembrane conductance regulator, and multidrug resistance-associated protein (17, 21, 32, 35). We considered the possibility that the relatively high levels of PE (40% of total disc lipid is PE) and/or docosahexaenoic acid (37% of ROS fatty acids) may provide a more favorable environment for ABCR. This was investigated by determining the effect of added DOPE, SDPE, DOPC, and SDPC on the ATPase activity of ABCR reconstituted in brain lipid. Both DOPE and SDPE increased the ATPase activity of ABCR in brain lipid, whereas DOPC and SDPC decreased the activity, indicating that a high PE content enhances the basal and retinal-stimulated ATPase activity of ABCR. The decrease in activity observed with added PC lipids can be explained in part by the effective dilution of endogenous brain lipid PE by added PC lipids. The importance of PE in retinal activated ATPase activity of ABCR is underscored by the finding that ABCR reconstituted in pure PC lipid vesicles is largely devoid of retinoid activation. This indicates that the coupling of retinoid binding within the membrane domain of ABCR to the ATPase activity of the nucleotide binding domains of ABCR requires a lipid environment rich in PE. The lipid environment is known to influence the ATPase and drug binding activities of P-glycoprotein (18, 19, 36). In this case, the phospholipid polar head group is less important because both egg PC and dipalmitoyl-PE stimulate P-glycoprotein ATPase activity, whereas egg PE does not. Loo and Clarke (37) have also reported that the nucleotide binding domains of P-glycoprotein interact with the transmembrane domains. High PE content is known to destabilize the lipid bilayer through hexagonal II phase formation (17). The increased accessibility of ABCR for ATP in such membrane sheets may also contribute to the increased basal ATPase activity observed in lipids containing a high PE content.

Earlier studies have shown that all-trans-retinal derived from photobleached rhodopsin reacts with PE in disc membranes to produce the Schiff base conjugate, N-retinylidene-PE (25, 38). This raises the possibility that either free retinal or N-retinylidene-PE is responsible for stimulation of ATPase activity of ABCR. In an effort to resolve this issue, we measured the effect of added N-retinylidene-PE on the ATPase activity of reconstituted ABCR. N-Retinylidene-PE, like retinal, resulted in over 3-fold stimulation of ATP hydrolysis. However, HPLC analysis following the addition of either free retinal or N-retinylidene-PE to brain lipid membranes suggests that an equilibrium is established between free retinal and N-retinylidene-PE, thereby complicating the identification of the substrate responsible for ATPase activation of ABCR. We have attempted to resolve this issue by determining whether N-retinyl-PE, the stable reduced form of N-retinylidene-PE, can stimulate the ATPase activity of ABCR. This compound, however, failed to stimulate the ATPase activity of ABCR. The identity of the retinoid substrate responsible for ATPase activation of ABCR was also investigated by measuring the effect of free retinal and retinylidene-PE on the ATPase activity of ABCR reconstituted into pure PC vesicles. However, neither retinoid stimulated ATP hydrolysis by ABCR in this lipid environment. Although this experiment failed to identify the retinal derivative responsible for ATPase activation of ABCR, it did reveal that such activation requires membranes containing substantial amounts of PE. This strongly suggests that PE is required to couple the binding of specific retinoids within the transmembrane domain of ABCR to the ATPase activity of the nucleotide binding domains.

Several indirect observations now support the view that ABCR may function to make retinal more accessible to all-trans-retinol dehydrogenase by either flipping N-retinylidene-PE from the luminal to the cytoplasmic side of the disc membrane or extruding retinal from the disc membrane. Substances that activate the ATPase activity of P-glycoprotein and other ABC transporters are also substrates for transport by these proteins. By analogy, activation of the ATPase activity of
ABCR by retinal compounds suggests that these physiological compounds may also be actively transported across disc membranes by ABCR. Patients with Stargardt disease-linked mutations in ABCR are known to exhibit delayed dark adaptation and accumulate diuretinopyridinium (A2E) compounds in the form of lipofuscin (26, 39–42). These characteristics can be explained on the basis of a diminished rate of 11-cis-retinal regeneration and accumulation of retinal-PE derivatives in disc membranes. Finally, recent studies indicate that abcr knockout mice also show reduced dark adaptation kinetics, elevated levels of protonated N-retinylethidene-PE, and accumulation of A2E compounds upon prolonged exposure to light (23). Efforts are now under way to directly identify the substrate for ABCR and the mechanism of substrate transport across disc membranes.

Unlike most cells, rod photoreceptors contain similar levels of ATP and GTP. Therefore, it was of interest to determine whether ABCR can catalyze GTP as well as ATP hydrolysis. Studies carried out earlier revealed that the kinetics of ATP and GTP hydrolysis by purified ABCR are similar. Moreover, basal ATPase activity is stimulated by similar concentrations of retinal. These results are consistent with earlier photoaffinity labeling studies showing that ABCR binds both ATP and GTP (2). GTP is a poor substrate for P-glycoprotein (18, 34) and does not support taurocholate transport by the bile salt transporter, MDR1. It remains to be determined whether GTP hydrolysis is able to support the active transport function of ABCR.

Finally, we have demonstrated that ABCR can be expressed in COS-1 cells. The purified and reconstituted protein exhibits basal and retinal-stimulated ATPase activity similar to ABCR from native ROS membranes. This provides strong evidence that the ATPase activity observed in immunoaffinity-purified ABCR from ROS is due to ABCR and not a retinal-sensitive ATPase contaminant. This cell expression system should be useful in the structure-function analysis of ABCR and understanding how selective mutations in ABCR cause Stargardt disease and related retinopathies.

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