Subunit Structure of Rod cGMP-Phosphodiesterase*

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The rod cGMP phosphodiesterase (PDE) is the G-protein-activated effector enzyme that regulates the level of cGMP in vertebrate photoreceptor cells. Rod cGMP PDE is generally viewed as a heterotrimeric protein composed of catalytic α and β subunits (~90 kDa each) and two copies of the inhibitory subunit γ (~10 kDa). However, the possibility that rod PDE could exist as distinct isoforms, such as αγ2 and ββ2γ has not been ruled out. We have studied this question using cross-linking of PDE subunits with maleimidobenzoyl-N-hydroxysuccinimide ester and para-phenyldimaleimide. The cross-linking resulted in major products with molecular mass of 118 and 135 kDa, a doublet at ~150–150 kDa, and a doublet at ~210–220 kDa. Cross-linked products were analyzed using polyclonal-specific anti-PDEαβ, anti-PDEα, anti-PDEβ, or anti-PDEγ antibodies. The anti-PDEα and anti-PDEαβ antibodies recognized all the cross-linked products, whereas anti-PDEβ and anti-PDEγ antibodies did not interact with the 150-kDa band, indicating that the composition of this band is most likely αα. Similar analysis of cross-linked products of trypsin-treated PDE preparations revealed bands that are likely formed by PDEβ subunit. The molecular size of holo-PDE and trypsin-activated PDE were studied using analytical ultracentrifugation in order to determine if oligomerization of PDE could account for the cross-linking of identical PDE subunits. The sedimentation analysis of both holo-PDE and ta-PDE revealed homogenous samples with molecular masses of ~220 and ~150 kDa, respectively. These results indicate that PDE is likely a mixture of the major species αβγ2, minor species ααγ2, and possibly ββγ2. Our data are consistent with the detection of low PDE activity in the rd mouse, which lacks any functional PDEβ subunit.

Photoreceptor rod and cone PDEs1 (PDE6 family) are members of a broad group of cGMP-binding PDEs that have cGMP-binding sites distinct from the sites of cyclic nucleotide hydrolysis (Charbonneau, 1990). Other members in this group are cGMP-stimulated PDE (PDE2 family), which hydrolyzes both cAMP and cGMP, and cGMP-binding, cGMP-specific PDE (PDE5 family) (Beavo, 1995; McAllister-Lucas et al., 1993). Bovine rod cGMP PDE is composed of two tightly bound catalytic PDEα and PDEβ (~90 kDa each) subunits and two identical inhibitory PDEγ subunits (10 kDa) (Baehr et al., 1979, Deterre et al., 1988). The primary structure of all three rod PDE subunits has been determined (Ovchinnikov et al., 1986; Ovchinnikov et al., 1987; Lipkin et al., 1990). The PDEα and PDEβ subunits have a high degree of homology (72%), and each contains a catalytic domain in the C-terminal part of the molecule (amino acids 555–790) that is highly conserved among all known cyclic nucleotide phosphodiesterases (Charbonneau et al., 1986; Lipkin et al., 1990). The PDEαβ catalytic domain contains the consensus sequence -NXXD- that specifies the guanine ring binding, a Mg2+ binding element DXXG, and a glycine-rich loop similar to the cAMP-binding site in the catalytic activator protein (Charbonneau et al., 1990; Li et al., 1990; Lipkin et al., 1990). Two noncatalytic cGMP-binding sites (Kd values 0.2–2.0 μM) were originally identified in frog rod PDE (Yamazaki et al., 1980). Later, noncatalytic cGMP-binding sites were also shown to be present in bovine PDE, however, with an affinity for cGMP much greater than that for frog PDE (Gillespie and Beavo, 1989). Analysis of the PDEα and PDEβ sequences has shown that these sites are located in the respective N-terminal regions. The N-terminal half of PDEα and PDEβ, as well as other members of cGMP-binding PDEs, contain two internally homologous regions (repeats) (Charbonneau et al., 1990; Li et al., 1990; Lipkin et al., 1990). These regions (I, amino acids 89–251 and II, amino acids 295–464 for PDEβ) are likely noncatalytic cGMP-binding sites (Charbonneau et al., 1990). Both regions have structural elements for cGMP binding. In addition, region II of the repeat has a weak degree of homology with cAMP/cGMP-binding sites on cAMP/cGMP-dependent protein kinases (Li et al., 1990; Lipkin et al., 1990). It appears, however, that the cGMP-binding domains of PDEs are unrelated to those of the catalytic gene activator protein and the cGMP or cAMP-dependent protein kinases (Shabb and Corbin, 1992). The C termini of both catalytic subunits contain the recognition site CXXX for post-translational prenylation and carboxymethylation. The C terminus of PDEα is farnesylated, whereas the C terminus of PDEβ is geranyl-geranylated (Ong et al., 1989; Anant et al., 1992). These modifications are responsible for PDE membrane attachment (Ong et al., 1989; Catty and Deterre, 1991).

It is commonly believed that the PDE catalytic core is a heterodimer PDEαβ (Fung et al., 1990). However, recent findings indicate that homodimer isoenzymes PDEαα and PDEββ may also be present (Lipkin et al., 1990; Piriev et al., 1993). In addition, evidence suggests that cone PDE is a homodimer composed of two identical PDEα subunits (~90 kDa) (Gillespie...
and Beavo, 1988) that share >60% homology with PDE\(\alpha\) and PDE\(\beta\) (Lipkin et al., 1990). Likewise, both cGMP-binding PDE from bovine lung and cGMP-stimulated PDE from bovine heart appear to be homodimeric proteins (Thomas et al., 1990; Martin et al., 1982). Here, we study the subunit structure of rod cGMP-phosphodiesterase using cross-linking of PDE combined with analysis of cross-linked products with the subunit-specific antibodies and determination of PDE molecular size using analytical ultracentrifugation.

**EXPERIMENTAL PROCEDURES**

**Materials—**cGMP was purchased from Boehringer Mannheim. t-1-Tosylamide-2-phenylhydrochloride ketone-trypsin and soybean trypsin inhibitor were obtained from Worthington. Protein A-Sepharose was purchased from Pharmacia Biotech, Inc. All other chemicals were from Sigma or Fisher.

**Preparation of PDE, ta-PDE, and t-PDE—**Bovine rod outer segment membranes were prepared by the method of Paparnes and Dreyer (1974). PDE was extracted from bleached rod outer segment membranes as described previously (Bachr et al., 1979). The PDE-containing extract was concentrated by ultrafiltration using a YM-30 membrane (Amicon). PDE, activated with limited trypsin digestion to remove \(\gamma\)-subunit (ta-PDE), was prepared essentially as described in Artemyev and Hamm (1992). To obtain trypsin-treated PDE (t-PDE) containing equimolar amounts of 88- and 70-kDa polypeptides, concentrated PDE extract (1 mg/ml) was digested with trypsin (50 \(\mu\)g/ml for 2 h at room temperature. Proteolysis was carried out in 20 mM HEPES buffer (pH 7.5), containing 1 mM MgCl\(_2\), 100 mM NaCl, 1 mM dithiothreitol, and 25\% glycerol. The reaction was stopped by the addition of soybean trypsin inhibitor (300 \(\mu\)g/ml). PDE, ta-PDE, or t-PDE were purified by ion-exchange HPLC on a Synchropak AX-300 column (10 \(\times\) 0.41 cm) (SynChrom, Inc.) or a MonoQ column (Pharmacia). The column was equilibrated with 20 mM HEPES buffer (pH 7.4), containing 1 mM MgCl\(_2\) and 100 mM NaCl. Proteins were eluted with a NaCl gradient (100--800 mM). In some experiments PDE was additionally purified using HPLC gel filtration. Fractions containing PDE were collected and applied to an HPLC gel filtration column, Spherogel-TSK 3000SW (38–49) (Tosylamido-2-phenylethyl chloromethyl ketone–trypsin and soybean trypsin inhibitor were obtained from Worthington. Protein A-Sepharose 4B (37–49) was purchased from Pharmacia Biotech, Inc. All other chemicals were from Sigma or Fisher.

**Preparation of Anti-PDE Subunit-Specific Antibodies—**Anti-PDE\(\beta\)-specific antisera were obtained by immunizing rabbits with purified PDE\(\beta\) and PDE\(\gamma\) subunits that were separated by reversed-phase HPLC on a preparative Aquapore Octyl column (25 \(\times\) 1 cm) (Applied Biosystem). The purity and chemical formula of each peptide was confirmed by fast bombardment mass spectrometry and analytical HPLC.

**Preparation of Anti-PDE Subunit-Specific Antibodies—**Anti-PDE\(\beta\)-specific antisera were obtained by immunizing rabbits with purified PDE\(\beta\) and PDE\(\gamma\) subunits that were separated by reversed-phase HPLC on a preparative Aquapore Octyl column (25 \(\times\) 1 cm) (Applied Biosystem). The purity and chemical formula of each peptide was confirmed by fast bombardment mass spectrometry and analytical HPLC.

**Chemical Cross-linking of PDE, ta-PDE, or t-PDE with MBS and PDM—**The cross-linking was carried out in buffer A with PDE concentration (10 \(\mu\)g/ml) in a concentration range of 0.5–2.5 \(\mu\)M MBS (1–10 mM) in acetone or PDM (1–10 mM) in dimethylformamide was added to a final concentration of 50 \(\mu\)M. Cross-linking was allowed to proceed for 5–30 min at room temperature. The reaction was quenched by adding an excess of ethanolamine or \(\beta\)-mercaptoethanol. PDE cross-linked products were analyzed by SDS-PAGE and Western immunoblotting. In control samples, PDE was incubated for 5–30 min under the same conditions including addition of solvent) as in cross-linked samples, only without cross-linker added. The proteins were transferred to nitrocellulose (0.1 \(\mu\)m, Schleicher & Schuell) as described previously (Towbin et al., 1979). The antibody-antigen complexes were detected using \(^{125}\)I-protein A or goat anti-rabbit IgG conjugated to horseradish peroxidase and ECL reagent (Amersham Corp.). The immunoblots were then exposed using Kodak X-ray film.

**Analytical Ultracentrifugation of PDE and ta-PDE—**Sedimentation equilibrium experiments were carried out by the method of Yphantis (1964) in an AN-F rotor with double sector charcoal-filled Epon 12-mm centerpieces with sapphire windows. All protein samples were in 20 mM MOPS buffer (pH 7.5), 0.5 mM MgCl\(_2\), 100 mM NaCl, and 0.2 mM dithiothreitol. The absorbances at 280 nm (protein concentrations) of the holo-PDE and ta-PDE samples were 0.03 (~0.3 \(\mu\)M) and 0.09 (~1 \(\mu\)M), respectively. The rotor speed was 10,000 rpm at 6 °C. The distribution of the protein was monitored by measuring absorbance at 280 or 237 nm with a photoelectric scanner at a series of points across the cell, and baseline correction was made using absorbance scans at 500 nm. Equilibrium was established by the constancy of the scans over time in hours. The tracings were recorded by an IBM PC as digital data points of concentration distribution as a function of radial distance from the center of rotation, \(r\). The distribution of the protein was expressed as a plot of absorbance versus \(r^2\). The data were fit by the nonlinear fitting program, Nonlin, to appropriate models (Johnson et al., 1981). Briefly, the program fits for monomer molecular weight, virial coefficient, and association constant, if the data indicate a system undergoing self-association. In a nonideal system, the relation between absorbance and \(r\) is shown in Equation 1

\[
\Delta A = \sigma_1 + 2 \sigma_2 r^2
\]

where \(\Delta A\) is the absorbance of the \(i\)th component at a radius \(r\), \(\sigma_i\) is the total absorbance of all components, \(B\) is the colligative virial coefficient, and (see Equation 2)

\[
\sigma = \frac{M_i(1-n_p)n^2}{RT}
\]

where \(M_i\) is the molecular weight of the \(i\)th component, \(R\) is the gas constant, \(T\) is the absolute temperature, \(\nu\) is the partial specific volume, \(\rho\) is the density of solvent, and \(\omega\) is the angular velocity. In this study the values of \(\nu\) and \(\rho\) were assumed to be 0.74 and 1.0, respectively.

**RESULTS**

Chemical cross-linking had been already used to study PDE structure and function. In an earlier report Hingorani et al. (1988) used the bifunctional cross-linker MBS, which reacts with amino and sulfhydryl groups, and the sulfhydryl linker PDM to cross-link PDE subunits. Hingorani et al. (1988) have shown that these cross-linkers are most effective in PDE cross-
linking, whereas amino-reactive homobifunctional cross-linkers, such as dimethyl suberimidate, cross-link PDE subunits poorly. Therefore, we selected MBS and PDM as cross-linkers for our experiments. However, lack of PDEα, -β, and -γ subunit-specific antibodies did not allow Hingorani et al. to identify the subunit composition of observed cross-linked products. The only study where cross-linked products between PDE catalytic subunits were analyzed with subunit-specific antibodies came to the conclusion that PDE catalytic subunits interact with identical subunits and PDEγ but do not interact with each other (Muradov et al., 1990). We have utilized chemical cross-linkers MBS and PDM with a panel of PDE subunit-specific antibodies to further study this question.

Analysis of Cross-linked Products of Holo-PDE—Chemical cross-linking of holo-PDE with either MBS or PDM resulted in the formation of products with similar molecular masses (Fig. 2). However, the relative amount of each product generated with the cross-linkers was different, and the efficiency of the cross-linking with PDM was much lower than with MBS. The molecular masses of cross-linked products observed after SDS-PAGE in 5% gel are as follows: 100 kDa, 140 kDa, a doublet at 180–190 kDa, and a doublet at 210–220 kDa. There are additional minor bands in the 225–230-kDa range (Fig. 2). These results are in agreement with the data of Hingorani et al. (1988). Increasing the time of cross-linking typically led to a larger proportion of the higher molecular mass products, whereas the cross-linking pattern did not appear to depend significantly on the concentration of PDE (0.5–2.5 μM) (data not shown). Such results suggest that the cross-linking reflects the formation of relatively stable PDE complexes in solution.

The composition of the cross-linked products was tested using anti-PDEαβ, anti-PDEα, or anti-PDEβ antibodies. Anti-PDEαβ and anti-PDEα recognized all of the cross-linked products (Figs. 2 and 3). The antibody recognition of the bands at 160-kDa band contain the PDEα subunit (Fig. 4). The 115-kDa band appears to be a 160-kDa protein. An additional minor band at 115 kDa was resolved using an 8% gel. This band also appears to react only with anti-PDEα antibodies. All other bands again were recognized with anti-PDEα and anti-PDEβ antibodies (Fig. 3). Western blotting with anti-PDEγ antibodies indicate that all PDE cross-linked products except the 160-kDa band contain the PDEγ subunit (Fig. 4). The composition of this band is most likely αα, while the 115-kDa band appears to be a αγγ cross-linked product. Low level of cross-linking of two PDEγ subunits to one catalytic subunit was also seen previously (Fung et al., 1990) and may indicate the close proximity of the PDEγ subunits in the PDE holo-enzyme.

Formation of the αα cross-linked product was rapid (within 5 min) and correlated with the formation of the αγ and βγ bands. That the αα cross-linked product does not contain the γ subunit may indicate that its formation occurs even faster than the cross-linking between PDE catalytic subunits and PDEγ. Thus, it appears that PDEα subunits interact with each other in solution. Cross-linking of ta-PDE and t-PDE—ta-PDE was obtained using limited trypsin digestion of holo-PDE in order to strip PDEγ subunits from the catalytic subunits. Cross-linking of ta-PDE (Fig. 5, lane 1) with MBS resulted in the formation of major products migrating at molecular masses of ~175 kDa and a doublet at 200 and 205 kDa as well as minor cross-linked products at ~120 and ~150 kDa (Fig. 5, lane 2). The
 Isoforms of Rod cGMP-Phosphodiesterase

minor products are not well seen on a Coomassie-stained gel; however, they are visualized with anti-PDEαβ and anti-PDEα antibodies. Anti-PDEαβ antibody recognized all the ta-PDE cross-linked products (Fig. 5). Anti-PDEα antibodies reacted with all bands, except probably the 200-kDa polypeptide. Anti-PDEαβ antibody did not recognize the cross-linked product at ~150 kDa (Fig. 5B). Interestingly, the yield of the 150-kDa αβ product is low after cross-linking of ta-PDE. A very weak signal of anti-PDEβ antibody at ~110–120 kDa might be nonspecific.

It has been previously reported that proteolysis of PDE with trypsin leads to the formation of a 70-kDa fragment (Hurley and Stryer, 1982; Catty and Deterre, 1991). Recently, we have demonstrated that trypsin cleaves after Lys-146 and Lys-147 of PDEβ subunit producing N-terminally truncated PDEβ polypeptide (Artemyev et al., 1996). Results of cross-linking of tPDE containing equimolar amounts of 88 and 70-kDa polypeptides are shown in Fig. 5A (lanes 3 and 4). This cross-linking produced products with molecular masses of ~75, 95, 120, 125, 170, 180, and 205 kDa. Anti-PDEαβ antibodies recognized all of the cross-linked bands. Anti-PDEα antibody reacted with the 95-, 120-, 125-, 170-, and 205-kDa bands (Fig. 5A). t-PDE was not recognized by anti-PDEβ antibodies since it lacks N-terminal region of PDEβ subunit. N-terminal tryptic fragment(s) of PDEβ appear to remain at least partially bound to the catalytic core of PDE (Artemyev et al., 1996). Therefore, the ~75- and ~95-kDa products most likely are cross-links of N-terminal fragment of PDEβ with the 70-kDa PDEβ polypeptide and 88-kDa PDEα, respectively. This cross-linked fragment (~5 kDa) is smaller than the full-length tryptic fragment of PDEβ (~16 kDa), and it lacks the anti-PDEβ antibodies recognition site because the antibody did not recognize the ~75- and ~95-kDa bands. The formation of two cross-linked products at ~120 and 125 kDa correlated with the presence of the 70-kDa PDEβ polypeptide in tPDE preparations. The ~120-kDa band appear to be a cross-link between PDEα and the 70-kDa PDEβ. This band is also detected as a very minor product after cross-linking of ta-PDE which reflects very low levels of 70-kDa band in ta-PDE (Fig. 5). The 125-kDa band could represent a product of cross-linking between PDEα, 70-kDa PDEβ, and the N-terminal polypeptide of PDEβ attached to PDEα or 70-kDa PDEβ.

Analysis of higher molecular weight cross-linked products of tPDE indicates that the 180-kDa band might contain only PDEβ subunit. This band is readily recognized by anti-PDEαβ, but seemingly it does not react with anti-PDEα antibody (Fig. 5). The 170-kDa product could be either a cross-link of only PDEα subunits or a cross-link between PDEα and PDEβ subunits. Results of the cross-linking of PDE are summarized in Table I.

Analytical Ultracentrifugation of PDE and ta-PDE—One potential scenario of how identical PDE subunits can be cross-linked is oligomerization of PDE. Therefore, in the next set of experiments we examined the molecular size of PDE using analytical ultracentrifugation. This procedure was performed on holo-PDE as well as on ta-PDE. Limited proteolysis of PDE removes PDEγ subunits, the farnesylated C-terminal fragment of PDEα, and the geranyl-geranylated C terminus of PDEβ (Catty and Deterre, 1991). The molecular size analysis of tPDE was performed in order to determine if prenylation of the PDE catalytic subunits and/or the presence of PDEγ subunits could contribute to potential oligomerization (specific or non-specific) of PDE.

Fig. 6 shows that the data sets for holo-PDE and ta-PDE are consistent with nonassociating solutions in both samples. The goodness of fit is indicated by the random distribution of residuals, as shown in the insets. In A and B, the data are consistent with a homogenous molecular mass of 2.2 (1.1, 3.3) × 106 Da and 1.5 (1.3, 1.6) × 106 Da for holo-PDE and ta-PDE, respectively. The values in parentheses represent the limits within a

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**Western blots**

**Fig. 4. Immunoblotting of PDE cross-linked products with anti-PDEα and anti-PDEγ-specific antibodies.** Holo-PDE (1 μM) was cross-linked with MBS (50 μM), separated on 5% SDS-gel, transferred to nitrocellulose paper, and analyzed with anti-PDEα or anti-PDEγ antibodies. Lane 1, 2: PDE, 2. PDE cross-linked with MBS for 15 min. The anti-PDEγ antibody binds to all cross-linked products except the 160-kDa band (indicated with arrow).

**Fig. 5. Analysis of ta-PDE and t-PDE cross-linked products.** A, ta-PDE (lane 1) or t-PDE (lane 3) (1 μM each) were cross-linked with MBS (50 μM) for 5 min (lanes 2 and 4, respectively), separated on 6% SDS gel, transferred to nitrocellulose paper, and analyzed with anti-PDEαβ, anti-PDEα, or anti-PDEβ antibodies. B, segments from overexposed immunoblots as in A showing recognition of the 150-kDa band by the PDE subunit-specific antibodies after ta-PDE (lane 1) cross-linking with MBS (lane 2).
67% confidence level. The greater uncertainty in the holo-PDE data is mainly due to the lower concentration of protein loaded in the cell. Fitting of the data to models of associating species led to an increase in the sum of the squares of deviations, i.e. the models were not as appropriate. Thus, it can be concluded that under the experimental conditions and within the concentration range investigated, there is no detectable oligomerization of either holo-PDE or ta-PDE. This supports the idea that cross-linking between identical PDE subunits reflects the formation of a PDEα and potentially -ββ species rather than oligomerization of PDE.

**DISCUSSION**

Rod cGMP phosphodiesterase has been the subject of thorough investigation since it was shown to be a major effector enzyme in visual transduction of vertebrate photoreceptor cells. Early studies demonstrated that PDE is composed of two large catalytic α and β subunits and a small inhibitory γ subunit (Baehr et al., 1979; Hurley and Stryer, 1982). Later, it was conclusively demonstrated that holo-PDE contains two copies of PDEγ subunit per PDE molecule and that both PDEα and PDEβ subunits bind PDEγ (Deterre et al., 1988; Whalen and Bitensky, 1989; Fung et al., 1990). PDEα and PDEβ subunits share a high degree of homology and have similar molecular masses and physicochemical properties. They can be separated by low cross-linked 15% SDS-PAGE (Baehr et al., 1979), where they appear as equally stained bands. Therefore, it was assumed that PDE is a complex αβγ. Alternatively, PDE could be an equimolar mixture of the isoenzymes ααγ and ββγ or a mixture of all three potential isoforms with unknown proportions of αγ, βγ, and αβ subunits. Fung et al. (1990) have tested this possibility using PDEα-specific antibodies. Anti-PDEα antibodies immunoprecipitated PDE containing equimolar amounts of PDEα and -β subunits, indicating that the composition of PDE is αβγ. In contrast, Muradov et al. (1990) reported the formation of ααγ, ββγ but not αβ or αγ complexes on rod outer segment membranes. Thus, we began our studies on rod PDE subunit composition using cross-linking of holo-PDE, ta-PDE, and t-PDE with MBS and analysis of the cross-linked products with subunit-specific antibodies.

The results of cross-linking of holo-PDE indicate that both anti-PDEα and anti-PDEβ antibodies recognize the majority of cross-linked products. Additionally, we noted the formation of a ~150-kDa product (migrates as 140- and 160-kDa polypeptide on 5 and 8% PAGE, respectively), which contains only the PDEα catalytic subunit. This cross-linked product was not detected with anti-PDEγ antibodies. Thus, the composition of this band appears to be αα. The molecular mass of this product is slightly different than what might be expected (180 kDa), however; quite often products of chemical cross-linking migrate anomalously on SDS-PAGE. We estimate that the yield of the αα cross-linked product may account for approximately 10–15% of PDE complexes.

Cross-linking of ta-PDE and t-PDE have demonstrated that most of the cross-linked products are formed by both α and β subunits of PDE. However, these data also support the likelihood of the minor ββ isoform of PDE. The 180-kDa tPDE cross-linked product appears to contain only PDEβ subunit. The 200-kDa ta-PDE cross-linked product also might contain only PDEβ subunit. It seems that this band is not recognized by anti-PDEα antibodies; however, it is not clear if anti-PDEβ antibodies react with both bands in the 200/205-kDa doublet.

It is very unlikely that the cross-linking destroyed the epitope of anti-PDEα or anti-PDEβ antibodies leading to lack of recognition of certain bands with the subunit-specific antibodies. Anti-PDEα and anti-PDEβ antibodies were developed against corresponding regions of PDEα and PDEβ, but anti-PDEα antibodies readily recognize the αα product and anti-PDEβ antibodies recognize the products containing the β-subunit. 2) Two different chemical cross-linkers (amino-reactive and sulfhydryl-reactive) were used in this analysis. Our cross-linking experiments therefore suggest that PDE is likely a mixture of the αβγ (major species) and minor species ααγ and ββγ. We were unable to estimate PDE composition by immunoprecipitation as carried out by Fung et al. (1990), because our antibodies did not immunoprecipitate PDE. However, immunoprecipitation analysis may not be sufficiently accurate to detect minor PDE isoforms. This would explain the difference between our results and the conclusions of the earlier study by Fung et al. (1990).

Alternatively, the αα and ββ cross-linked products could be formed by oligomerization of PDE. The oligomeric structures of PDE would consist of more than two catalytic subunits, and in certain geometric arrangements two identical catalytic subunits might be cross-linked. Analytical ultracentrifugation of holo-PDE demonstrated a homogeneous sample with molecular mass of ~220 kDa. This result is consistent with a dimeric structure of PDE catalytic subunits and is in good agreement with earlier estimations (Baehr et al., 1979). Analytical ultracentrifugation demonstrated that ta-PDE has a molecular mass of ~150 kDa. The difference in molecular masses of holo-PDE and ta-PDE can be explained by the removal by limited trypsinization of the two PDEγ subunits and the prenylated C termini from PDE α and β subunits. No oligomerization of PDE or ta-PDE was detected at protein concentrations similar to those used in the cross-linking experiments. These data suggest that the prenylation of PDE catalytic subunits, which is responsible for the membrane attachment of PDE, does not cause any nonspecific oligomerization of PDE in solution.

The existence of the putative rod PDE ααγ and, perhaps, ββγ isoforms is consistent with the known structural and functional characteristics of closely related PDEs. Cone PDE most likely is a homodimer composed of two PDEα’ subunits (Gillispie and Beavo, 1988) that share ~60% homology with PDEα and PDEβ (Lipkin et al., 1990). Two PDEs, evolutionarily related to photoreceptor PDEs, cGMP-binding PDE from bovine lung and cGMP-stimulated PDE from bovine heart, are both probably homodimeric proteins. Limited chymotryptic proteolysis of these PDEs has localized the domain responsible for dimerization to a ~35-kDa fragment from the PDE N-terminal half (Thomas et al., 1990; Stroop and Beavo, 1991).
This fragment consists of two homologous noncatalytic cGMP-binding motifs with a linker region between them (Charbonneau et al., 1990; Thomas et al., 1990; Stroop and Beavo, 1991). It is likely that cGMP-binding PDE and cGMP-stimulated PDE have a common dimeric organization with photoreceptor PDEs. If so, the dimerization motifs of photoreceptor PDEs might be localized within the noncatalytic cGMP-binding repeats and the linker region. These regions are highly homologous among cone α and rod α and β subunits (>90%) (Li et al., 1990; Lipkin et al., 1990).

Expression of individual rod PDE catalytic subunits also supports the possibility of functionally active PDE αγ2 and ββγ2 isoforms. Recently, individual PDE catalytic subunits were expressed in human embryonic kidney cells (Piriev et al., 1993). PDE α and β subunits had low levels of PDE activity. This activity increased 6-fold when heterodimer PDEαβ was reconstituted from PDEα and PDEβ (Piriev et al., 1993). A similar increase in PDE activity was observed for PDEα and PDEβ co-expressed in Sf9/baculovirus system (Qin and Baehr, 1994). Different activities of PDE isoforms may merely reflect their stability, but they have potential to be functionally relevant in phototransduction.

An important implication for the PDE isoforms is provided by studies of the retinal degeneration of the rd mouse. This retinal degeneration affects predominantly the rod photoreceptors and leads to the destruction of the outer nuclear layer in the rd mouse retina. Progression of the retinal degeneration correlates with the elevation of cGMP levels in the rd retina.

**Fig. 6. Analytical ultracentrifugation of PDE and ta-PDE.** Equilibrium sedimentation analysis of PDE and ta-PDE was performed as described under “Experimental Procedures.” A, holo-PDE sample; B, ta-PDE sample. The lines show the best fit of the data sets to the nonassociating model. The insets show the residuals of the fitting.
but also how this affects PDE activity and stability. Subunits and mechanisms of PDE post-translational assembly (McLaughlin et al., 1992). The PDEβ mutations result in the absence of functional PDEβ subunit in the rd retina. Nevertheless, the rd mouse photoreceptors contain low PDE activity (Farber and Lolley, 1977), which is attributed to the PDEα subunit alone (Piriev et al., 1993). It also appears that mutations in the PDEβ gene are responsible for some human retinal diseases. Recessive mutations of the PDEβ gene have been found in patients with retinitis pigmentosa (McLaughlin et al., 1993). Therefore, it is important not only to learn more about the dimerization motifs of rod PDE catalytic subunits and mechanisms of PDE post-translational assembly but also how this affects PDE activity and stability. Studies of rd mouse suggest that the rod PDEα forms a functional αβγ enzyme with reduced activity in the rd retina. Our results indicate that the αγβγ and, potentially, βγPDE isoenzymes may exist as a normal minor subspecies in bovine rod photoreceptors. Further studies are needed to better understand functional implications of the putative PDE isoforms in visual transduction.

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