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The membrane binding domain of rod cGMP phosphodiesterase is posttranslationally modified by methyl esterification at a C-terminal cysteine

(phototransduction/guanine nucleotide-binding protein/protein methylation/proteolysis)

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ABSTRACT **Retinal rod cGMP phosphodiesterase (3′,5′-cyclic-GMP phosphodiesterase; EC 3.1.4.35; PDE), a key regulatory enzyme involved in visual excitation, is one of several outer segment membrane proteins that are carboxyl methylated in the presence of the methyl donor *S*-adenosyl-L-[^{3}H-*methyl*]methionine. By chromatographic analyses of the ^{3}H-methyl amino acid generated by exhaustive proteolysis of purified PDE, followed by performic acid oxidation of the digest, we have shown that this modification occurs at a C-terminal cysteine residue of the α subunit of this enzyme. When PDE is subjected to limited proteolysis with trypsin, a ^{3}H-methylated fragment of 1000 daltons or less is rapidly removed prior to the degradation of its inhibitory γ subunit. This small fragment remains membrane bound, whereas the bulk of the enzyme is released, indicating that a domain responsible for anchoring PDE to the membrane is located near the C terminus. Based on the C-terminal amino acid sequence of Cys-Cys-Val-Gln predicted from the α cDNA sequence, we conclude that PDE undergoes posttranslational modifications, including the proteolytic removal of two or three terminal amino acids, and methyl esterification of the α-carboxyl group of the terminal cysteine residue. We speculate that the sulfhydryl group of the methylated cysteine is also lipidated to mediate membrane binding. These modifications may play an important role in delivering the nascent PDE chains to the membrane and in correctly positioning the PDE molecule in the rod disks for phototransduction.**

Visual excitation in vertebrate retinal rods is mediated by a cGMP cascade that converts light into cellular responses (reviewed in ref. 1). An amplified signal is generated when photolyzed rhodopsin activates a retinal G protein (transducin), which, in turn, stimulates the activity of a cGMP phosphodiesterase (3′,5′-cyclic-GMP phosphodiesterase; EC 3.1.4.35; PDE). PDE, the key regulatory enzyme involved in this process, is composed of α (M_r = 90,000), β (M_r = 88,000), and γ (M_r = 10,000) polypeptides. In this multimeric form, the hydrolytic activity associated with α and β is inhibited by the γ subunit. PDE is activated when γ is removed by interaction with the GTP-bound form of transducin. The transient decrease in intracellular concentration of cGMP then leads to the closure of many cGMP-regulated cation channels in the plasma membrane, resulting in a decrease in Na^+ influx and hyperpolarization of the rod.

PDE is a peripheral membrane protein that can be eluted from disk membranes of the outer segments by extraction with low ionic strength buffers (2, 3) or by brief proteolysis with trypsin (4). However, unlike transducin, which is known to bind tightly to rhodopsin in a light-dependent manner, PDE appears to be associated with the lipid matrix (5). The mechanism by which PDE attaches to the surface of the disk membrane, however, remains to be elucidated.

Recently, we proposed that a class of membrane-associated proteins is modified by methyl esterification at the α-carboxyl group of an invariant cysteine residue following posttranslational proteolysis of three C-terminal residues (6). The same cysteine residue may also be lipidated to allow the attachment of these proteins to the membranes for signal transduction. An earlier study by Swanson and Applebury has shown that the α subunit of PDE is methyl esterified, although the site of this covalent modification was not determined (7). In this paper, we present evidence that PDE is indeed a member of this class of proteins possessing a methylated C-terminal cysteine. We further show that the methylated region of PDE is crucial in membrane binding.

EXPERIMENTAL PROCEDURES

Materials and Assays. The preparation of rod outer segment (ROS) membranes from frozen bovine retinas (J. A. Lawson, Lincoln, NE) and reconstituted membrane vesicles containing purified rhodopsin and egg yolk phospholipids (rhodopsin:lipid = 1:125) was carried out as described (8). SDS/PAGE was performed by the method of Laemmli (9). Electrophoretic transfer blotting (Western blotting) was performed according to the procedure of Towbin *et al.* (10) using ^{125}I-labeled protein A to detect the immunoreactive peptides. Protein concentrations were determined by Coomassie blue binding (11) using gamma globulin as a standard. Analysis of methyl ester linkages by alkaline hydrolysis was performed by the vapor-phase equilibration assay (12).

Purification of Methylated PDE. Methylation of proteins in ROS was carried out by a modified procedure of Swanson and Applebury (7). Briefly, purified ROS containing 25 mg of rhodopsin were incubated with *S*-adenosyl-L-[^{3}H-*methyl*]-methionine ([^{3}H]AdoMet) (New England Nuclear; 0.7 μM, 79.6 Ci/mmol; 1 Ci = 37 GBq) in 10 ml of buffer A (50 mM hydroxyethylpiperazineethanesulfonic acid, pH 7.0/100 mM NaCl/5 mM $MgCl_2$/0.1 mM dithiothreitol) for 2 hr at 37°C. Following methylation, the membranes were washed three times with 40 ml of buffer A to remove the residual [^{3}H]AdoMet and extracted twice with 10 ml of the hypotonic buffer (5 mM Tris, pH 7.5/0.5 mM $MgCl_2$/1 mM dithiothreitol) as reported (13). These extracts, which contained methylated PDE and other peripheral membrane proteins, were used as a source for PDE purification by successive chromatographies on DEAE-Sephacel and Bio-Gel P-200 according to a

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Abbreviations: AdoMet, *S*-adenosyl-L-methionine; ROS, rod outer segment(s); PDE, retinal rod cGMP phosphodiesterase; TPCK-trypsin, L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin; α, β, and γ, subunits of cGMP phosphodiesterase.

modified procedure (13) of Baehr et al. (2). Alternatively, the extract was concentrated and used for membrane binding experiments.

Total Enzymatic Digestions of ^{3}H-Methylated PDE. The protocol of Ota and Clarke (14) was employed to enzymatically convert ^{3}H-methylated PDE to their amino acid constituents with proteases. Labeled PDE (45–150 μg of protein) was digested with 1.5 μg of diphenyl carbamoyl chloride-treated bovine pancreatic trypsin (Sigma; 7500–9000 *N*-benzoyl-L-arginine ethyl ester units/mg of protein) in 8.3 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.4/67 mM NaCl/0.67 mM $MgCl_2$/0.33 mM dithiothreitol for 30 min at 37°C in a total volume of 90 μl. The reaction was quenched by the addition of 85 μl of 10% (wt/vol) trifluoroacetic acid. After removing the insoluble residue by centrifugation, the supernatant was dried by lyophilization. This sample was then digested with 30 μg of microsomal leucine aminopeptidase (Sigma, type IV-S; 0.6 μmol of L-leucine-*p*-nitroanilide hydrolyzed per min at pH 7.2 and 37°C) and 28 μg of prolidase (Sigma; 5.2 μmol of Gly-Pro hydrolyzed per min at pH 8.0 and 37°C) in 92 mM 2-bis(2-hydroxylethyl)amino-2-(hydroxylmethyl)-1,3-propanediol buffer (pH 6.0) containing 4 mM $CaCl_2$ and 4 mM $MnCl_2$ in a total volume of 240 μl. The digestion was continued for 220 min at 40°C and then quenched with 50 μl of 10% (wt/vol) trifluoroacetic acid. This material was lyophilized and oxidized with 0.8 ml of performic acid for 23 hr at 4°C (14).

Localization of the Membrane Binding Domain by Tryptic Digestion. The binding of PDE to membranes was initiated by adding 320 μl of hypotonic PDE extract (1.1 mg of protein per ml) to 190 μl of reconstituted membrane vesicles (2.87 mg of photolyzed rhodopsin per ml); this was followed by adding 56 μl of hypertonic buffer (1 M Tris, pH 7.5/1 M NaCl/20 mM $MgCl_2$/10 mM dithiothreitol). The mixture was incubated on ice for 15 min, and the membranes containing bound PDE were separated from soluble proteins by centrifugation in a Beckman Airfuge at 63,000 × *g* for 3 min. The pellet, which contained ≈50–75% of the total PDE added, was resuspended to 1 mg of rhodopsin per ml in buffer A and digested with 5 μg of L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (TPCK-trypsin) per ml. The reaction was terminated by adding 8.6 μl of soybean trypsin inhibitor (0.6 mg/ml) to 95 μl of the mixture. The membranes were then pelleted at 63,000 × *g* for 3 min, solubilized in electrophoresis sample buffer, and analyzed by SDS/PAGE. One set of gels was stained with Coomassie blue and the proteins were quantitated by densitometric scanning. Another identical set of unstained gels was cut into 3-mm slices, digested in 0.5 ml of 20% hydrogen peroxide at 65°C, and assayed for radioactivity.

RESULTS

Methylation Occurs on a C-Terminal Cysteine Residue. Bovine ROS were incubated with [^{3}H]AdoMet under conditions where the labeled methyl group was incorporated into PDE in methyl ester linkages (7). After extraction of methylated PDE from ROS with low ionic strength buffer and further purification by chromatography on a DEAE-Sephacel column (2, 13), analysis by SDS/PAGE showed that >70% of the total protein was present as PDE. Moreover, scintillation spectroscopy of the gel slices indicated that 85% of the total radioactivity was associated with the α subunit of PDE, consistent with the result of Swanson and Applebury (7). When the radioactivity corresponding to the PDE band was determined by vapor-phase equilibration assay (12), >90% of the radioactivity was found to be present in methyl ester linkages. We estimate that the initial stoichiometry of the *in vitro* methylation of PDE is approximately one or two methyl groups per 100 molecules; longer incubations with [^{3}H]AdoMet do not significantly increase this level. The ^{3}H-methyl ester of the extracted PDE, however, is not stable, and the radioactivity decreases markedly over a period of several days during the PDE purification. The stoichiometry of methylation of the purified product is therefore less.

The DEAE-purified preparation of PDE was enzymatically converted to its amino acid constituents by the combined action of trypsin, leucine aminopeptidase, and prolidase, followed by performic acid oxidation, a treatment that quantitatively cleaves thiol ester-linked lipids and may partially cleave thiol ether-linked lipids (14). If a ^{3}H-methylated C-terminal cysteine is present, it will be released as free cysteic acid ^{3}H-methyl ester. This digest was mixed with a synthetic standard of L-cysteic acid methyl ester (15) and then fractionated on an amino acid analysis column. As shown in Fig. 1*A*, a major peak of radioactivity, accounting for 19.1% of that present in methyl esters in the PDE preparation, was found to elute in the position of the cysteic acid methyl ester standard. The 19.1% yield probably represents a minimum estimate of the content of C-terminal cysteinyl methyl esters because of spontaneous hydrolysis of the methyl ester during the isolation procedure, esterolytic activity of the proteolytic enzymes, and potential incomplete cleavage of thiol ether-linked lipids from the cysteine residue. The radioactive material eluting at 9–11 min and 15–16 min has not been

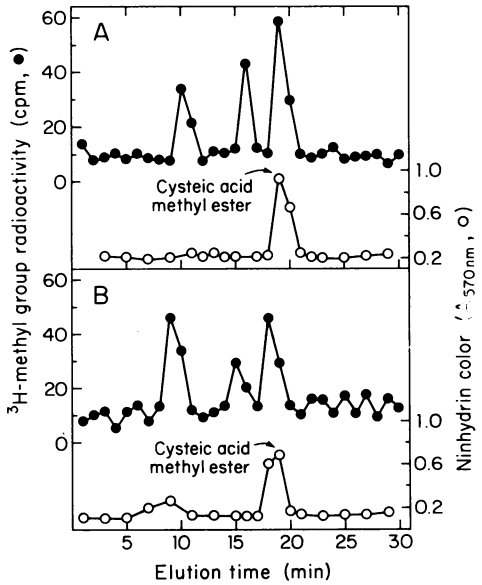

FIG. 1. Amino acid analysis of enzymatic digests of ^{3}H-methylated PDE. Two preparations of ^{3}H-methylated PDE were digested sequentially with proteolytic enzymes and oxidized with performic acid. The oxidized digest was lyophilized, resuspended in 100 μl of water and 200 μl of pH 2.2 sodium citrate sample buffer (Pierce; 0.2 M in sodium ion), and mixed with 4 μmol of L-cysteic acid methyl ester standard (15). This mixture was fractionated by amino acid analysis on a column of Beckman AA-15 sulfonated polystyrene resin [0.9 cm (diameter) × 54 cm] at 56°C in a pH 3.25 sodium citrate buffer. One-minute fractions (1.1 ml) were collected and assayed for total radioactivity (●) by counting 100 μl (*A*) or 250 μl (*B*) of each fraction in 5 ml of scintillation fluor. The elution position of the cysteic acid methyl ester standard was determined by manual ninhydrin analysis of 20-μl (*A*) or 50-μl (*B*) aliquots of column fractions as described (16) (○). (*A*) Digestion products obtained from 150 μg of PDE purified through the DEAE column. From an initial radioactivity of 4470 cpm in methyl ester linkages, 3800 cpm were recovered as methyl esters after the trypsin digestion and 3070 cpm were recovered after the leucine aminopeptidase/prolidase steps. (*B*) A similar analysis of ^{3}H-methylated PDE that had been further purified by gel filtration (45 μg of protein, 1760 cpm as methyl esters). In each case, a major peak of radioactivity is found to coelute with the cysteic acid methyl ester standard at 18–20 min.

identified and may represent incomplete cleavage products. Under these conditions, aspartic acid β-methyl ester elutes at 60 min and glutamic acid γ-methyl ester elutes at 90 min.

The identity of cysteic acid ^{3}H-methyl ester from the digestion was further confirmed by demonstrating that all of the radiolabel in the amino acid analysis peak also comigrated with the cysteic acid methyl ester standard when fractionated by Sephadex G-15 gel filtration chromatography or by thin-layer electrophoresis at pH 6.5 (data not shown) as well as when fractionated in two thin-layer chromatography systems (Fig. 2). To assure that the radioactivity isolated as cysteic acid methyl ester originated from PDE, the labeled enzyme was further purified on a Bio-Gel P-200 gel filtration column (13). The protein and radiochemical purity of this preparation was ≈95% (Fig. 3 *Upper*). This purified PDE was similarly digested and fractionated on an amino acid analysis column (Fig. 1*B*). A nearly identical pattern of radioactivity was found and the yield of cysteic acid ^{3}H-methyl ester, determined as described above, was 16.2%. These results show that methylation of PDE occurs at a C-terminal cysteine of the α chain.

Limited Tryptic Digestion Selectively Removes the Carboxyl Methylated Region of PDE. Tryptic digestion of purified PDE rapidly removed the carboxyl methylated region of the α subunit. As shown in Fig. 3 *Lower*, a 5-min treatment at a trypsin/PDE ratio of 1:100 resulted in the disappearance of

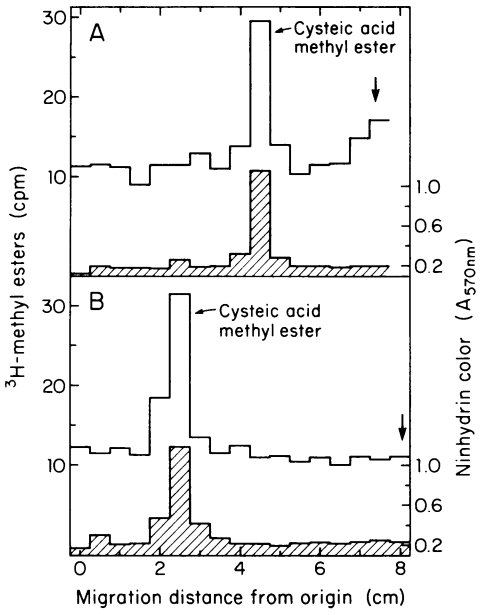

FIG. 2. Confirmation of the identification of cysteic acid ^{3}H-methyl ester from enzymatic digests of ^{3}H-methyl esterified PDE in two thin-layer chromatography systems. The cysteic acid methyl ester peak from the amino acid analysis column (Fig. 1, upper trace) was pooled and desalted on a 1.5 cm (diameter) × 85 cm column of Sephadex G-15 eluted at room temperature in 0.1 M acetic acid. Fractions (2.2 ml) were collected and analyzed for radioactivity and ninhydrin-reactive material as in Fig. 1. All of the radioactivity coeluted with the peak of the cysteic acid methyl ester standard (data not shown). Fractions were lyophilized and resuspended in a minimal volume of water. A portion of each sample was applied to a cellulose sheet and subjected to thin-layer chromatography at room temperature in solvents of 1-propanol/water, 7:3 (vol/vol) (*A*), or 1-butanol/acetic acid/water, 4:1:1 (vol/vol) (*B*), exactly as described (14). The amount of radioactivity in methyl ester linkages as [^{3}H]methanol was determined by a vapor-phase equilibration assay of base-treated 0.5-cm sections of the cellulose plate (14). The amount of L-cysteic acid (hatched bars) formed from the L-cysteic acid methyl ester standard in the nonvolatile fraction of these same sections was determined by ninhydrin assay (14). The position of the solvent front is marked with an arrow.

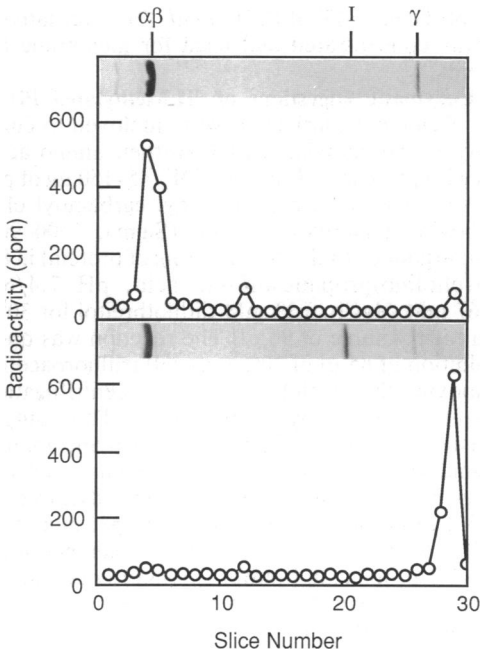

FIG. 3. Selective removal of the ^{3}H-methylated region of PDE by limited proteolysis with trypsin. Purified ^{3}H-methylated PDE (0.6 mg/ml) was digested with TPCK-trypsin for 5 min in 10 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.5/100 mM NaCl/2 mM $MgCl_2$/dithiothreitol at 22°C at a protease/substrate ratio of 1:100. The reaction was terminated by the addition of a 10-fold excess of soybean trypsin inhibitor over the TPCK-trypsin. Multiple samples of 6.5 μg of ^{3}H-methylated PDE (*Upper*) or 6.5 μg of digested ^{3}H-methylated PDE (*Lower*) were analyzed by SDS/PAGE. One set of gels was stained with Coomassie blue. Another identical set of unstained gels was cut into 2-mm slices, digested in 0.5 ml of 20% hydrogen peroxide at 65°C overnight, and assayed for radioactivity (○). The position of soybean trypsin inhibitor marker is denoted by the symbol I.

>90% of ^{3}H-methyl label from the α subunit, with concomitant appearance of radioactivity at the dye front position. Radioactivity was absent in the gel when a [^{3}H]methanol standard was subjected to SDS/PAGE, showing that the radioactive band at the dye front position was not methanol generated by ester hydrolysis. Under these cleavage conditions, the electrophoretic mobility of the α and β bands apparently remained unchanged, indicating that trypsin only removed a small peptide of probably <1000 daltons from the C-terminal region of the α subunit. Based on the small size of this ^{3}H-methylated peptide, the cleavage most likely occurs at either Lys-844 or Lys-853 (17), which will generate a fragment of 2 or 11 amino acid residues. These peptides do not contain aspartyl or glutamyl residues (17), further supporting the identification of the methylation site at the C-terminal cysteine.

The inhibitory γ subunit of PDE is known to be degraded by trypsin, resulting in a marked increase in phosphodiesterase activity (18, 19). We found that the selective removal of the C terminus of α by trypsin occurred rapidly and preceded the degradation of γ. As shown in Fig. 4, >85% of the radioactivity associated with the α band was released after a 5-min incubation at room temperature with trypsin. At this time point, <30% of the γ subunit was cleaved (Fig. 4 *Inset*), as demonstrated by a Western blot analysis of the cleaved PDE using anti-peptide antibodies against the N- and C-terminal regions of γ (19).

The C-terminal Region of the α Subunit Is Involved in Membrane Binding. A clue to the function of the methylated C-terminal region was provided by an earlier study of Wensel and Stryer (4), who proposed that trypsin releases PDE from

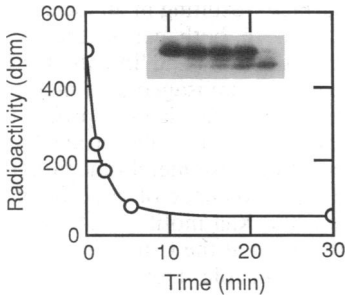

FIG. 4. Cleavage of the ^{3}H-methylated region precedes the degradation of the γ subunit. Purified ^{3}H-methylated PDE (0.45 mg/ml) was digested with TPCK-trypsin as described in the legend of Fig. 3. At indicated time points, 12 μl of the mixture was removed and the reaction was quenched with 6 μl of soybean trypsin inhibitor (0.09 mg/ml), followed by the addition of 6 μl of 4× electrophoresis sample buffer. Proteins in 18 μl of each sample were separated by SDS/polyacrylamide gels. The $\alpha\beta$ bands of PDE were excised, digested with 20% hydrogen peroxide, and assayed for radioactivity (○). Proteins in 4 μl of the same samples were analyzed on a Western blot using a mixture of anti-γ peptides (antiγ_{2-16} and anti-γ_{73-87}) and anti-PDE antiserum (18) and then detected with ^{125}I-labeled protein A. (*Inset*) Region of autoradiogram on which the γ subunit and its fragments were detected.

the ROS membranes by nicking either the α or β subunit. As shown by SDS/PAGE analysis of membrane pellets (Fig. 5), PDE extracted from ROS with low ionic strength buffer readily reassociated with reconstituted membrane vesicles composed of rhodopsin and egg yolk phospholipids (lane 2), consistent with the previous findings that the enzyme binds to the lipid matrix (5). In contrast, the binding of PDE was impaired after the extract was treated briefly with trypsin to remove the C-terminal region of the α subunit (lane 3).

Methylated PDE bound to rhodopsin-phospholipid vesicles was also released from the membrane when subjected to tryptic digestion (data not shown). When an unstained gel of this preparation was analyzed for the ^{3}H-methyl label, the radioactivity was found in the membranes and migrated at the dye front position between gel slice no. 20 and no. 23 (Fig. 6*A*), indicating that the small proteolytic fragment containing

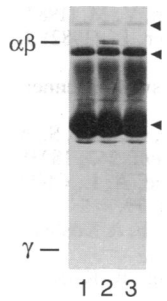

FIG. 5. Effect of tryptic removal of the ^{3}H-methylated region on PDE binding to membrane. A sample of 38 μl of hypotonic PDE extract (1.1 mg of protein per ml) was digested with 2 μl of trypsin (0.36 mg/ml) for 2 min at 22°C followed by the addition of 2 μl of soybean trypsin inhibitor (2 mg/ml) to terminate the reaction. Membrane binding was assayed by centrifugation after a 15-min incubation of trypsin-treated PDE (lane 3) with 25 μl of rhodopsin-phospholipid membranes (2.87 mg of rhodopsin per ml) and 8 μl of hypertonic buffer (1 M Tris, pH 7.5/1 M NaCl/ 20 mM $MgCl_2$/10 mM dithiothreitol). Controls consisting of membranes alone (lane 1) or intact PDE plus membranes (lane 2) were assayed under identical conditions. The membrane pellets were dissolved in electrophoresis sample buffer, and 18 μg of rhodopsin from each sample was analyzed by SDS/PAGE. Arrowheads indicate positions of rhodopsin monomer, dimer, and trimer.

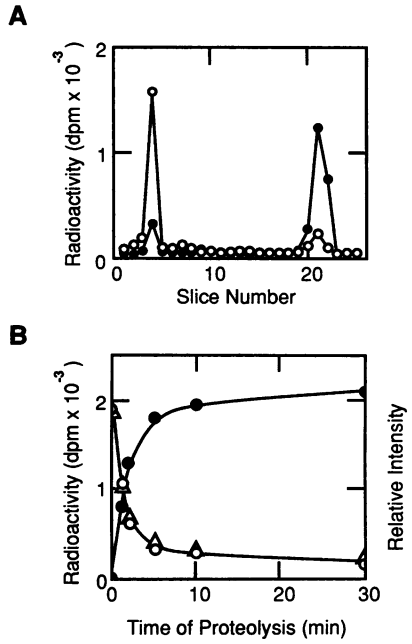

FIG. 6. Localization of the membrane binding domain to the carboxyl methylated region of PDE. (*A*) SDS/PAGE analysis of the radioactivity in the membrane pellets before (○) and after a 5-min tryptic digestion (●) of the membrane-bound ^{3}H-methylated PDE. (*B*) Decrease in the amounts of membrane-bound ^{3}H-methylated PDE, as quantified by protein (△) and radioactivity (○), and the corresponding increase in membrane-bound ^{3}H-methyl tryptic fragment (●).

the methylated C-terminal region of the α subunit is lipophilic.

Throughout the time course of tryptic digestion, the amounts of PDE associated with the membrane pellets, as determined from the densitometric scans of the Coomassie blue staining intensity of the $\alpha\beta$ bands, were in excellent correlation with the decrease in radioactivity of the membrane-bound α subunit, as well as the concomitant increase in radioactivity associated with the C-terminal fragment at the dye front (Fig. 6*B*). These findings strongly suggest that PDE is anchored to the membrane through the C-terminal region of the α subunit.

DISCUSSION

In this study we show that methylation of the PDE α subunit occurs at the α-carboxyl group of a C-terminal cysteine residue. This conclusion is based on amino acid analysis of proteolytic digests of methylated PDE as well as additional chromatographic analysis of the cysteic acid methyl ester product. The C-terminal cysteine residue is most likely the only target for methylation since almost all the radioactivity associated with the ^{3}H-methyl group can be released by limited proteolysis with trypsin without any observable change in electrophoretic mobility of the α chain. Although several fungal mating factor peptides (cf. ref. 20) and at least one polypeptide of bovine ROS in the molecular mass range between 23,000 and 29,000 daltons (14) have been shown to be methyl esterified at the C-terminal cysteine residue, to our knowledge, such a modification in a well-characterized enzyme has not been reported previously. The carboxyl methylation of the ras proteins (6, 15, 21), the nuclear lamin B protein (22), and a 23-kDa GTP-binding protein isolated from brain (23) may be additional examples of this type of modification.

For each of the methylated proteins described above in which the cDNA sequence has been determined, the deduced amino acid sequence predicts an invariant cysteine residue at the fourth position from the C terminus. We have proposed that the methylation of the α-carboxyl group of the cysteine residue of this class of proteins occurs after the posttranslational removal of three amino acid residues from the C terminus (6). So far, both the known C-terminal sequence Cys-Cys-Val-Gln deduced from the cDNA of the PDE α subunit (17) and our observation of methyl esterification of PDE at the cysteine residue support this model, although the possibility that only two amino acids are removed in this case cannot be ruled out. At this time, it is also not clear why only a small fraction of PDE molecules are methylated under our *in vitro* reaction conditions. Based on the model proposed for the ras proteins (21), methyl esterification probably occurs shortly after the synthesis of the polypeptide and one would thus expect that most PDE molecules in membranes already have been methylated. The *in vitro* methylation seen here may represent the modification of the small fraction of molecules that have lost their methyl groups, perhaps by spontaneous hydrolysis reactions. Further studies will be needed to define the relative populations of methylated and unmethylated PDE molecules, the factors that control these populations, and how the activities of these species may differ.

In addition to being modified by methyl esterification, the sulfhydryl group of C-terminal cysteine residues of this class of proteins may also be lipidated by the addition of palmitic acid in a thiol ester linkage or farnesyl derivatives in a thioether linkage (21, 24). In ras proteins, this invariant cysteine residue is essential in membrane localization (25) and appears to be farnesylated in a biosynthetic step that occurs prior to the attachment of a palmitoyl group to a nearby cysteine residue (21). Although we have no direct evidence showing that the C-terminal methylated cysteine of the α subunit is similarly modified by lipidation, our findings that carboxyl methylation and membrane binding properties are localized to a very small region of the C terminus are in agreement with this type of modification. Consistent with this interpretation is our observation that over a period of 24 hr the ability of PDE to reassociate with the reconstituted membranes decreases by 40–50%, whereas the phosphodiesterase activity remains stable (data not shown). A similar decrease in light-activated phosphodiesterase activity following reassociation of extracted PDE and transducin with ROS disk membranes has been reported (26). These results suggest that a relatively labile lipophilic moiety rather than an intrinsic hydrophobic primary structure may be involved in membrane anchoring.

It is interesting to speculate that posttranslational processing of the α subunit at the C terminus might play two important roles in positioning the PDE molecule in the disk membranes for signal transduction. (*i*) Lipidation and methylation of the α subunit might serve to direct the newly synthesized PDE to a membrane target, where it is assembled with other retinal proteins and then delivered to the outer segment compartment of the rod. (*ii*) The lipophilic C-terminal domain might be essential for orienting the PDE molecule on the disk membrane and in bringing PDE close to rhodopsin and transducin. Interactions between these transduction proteins would then proceed by lateral diffusion on the membrane surface, resulting in an increase in the rate of photoactivation (27). In both cases, the posttranslational modifications by methylation and lipidation of the C-terminal cysteine residue can be envisaged as a membrane-directing signal to place the PDE molecule in its proper locations, thus enabling it to fully participate in phototransduction. Whether this is a common pathway for membrane targeting remains to be evaluated, but the existence of large families of proteins with the Cys-Xaa-Xaa-Xaa motif (6, 14, 21) raises the possibility that methylation at the C-terminal cysteine may play a much more important role than previously suspected.

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