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Molecular cloning and expression of human myocardial cGMP-inhibited cAMP phosphodiesterase

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ABSTRACT **We have cloned a cDNA for a myocardial cGMP-inhibited cAMP phosphodiesterase (cGI PDE) from a human heart cDNA library in λ Zap II. The open reading frame [3.5 kilobases (kb)] of cDNA clone n.13.2 (7.7 kb) encodes a protein of 125 kDa. In Northern blots of total human ventricle RNA, a single mRNA species (8.3 kb) hybridized with a 4-kb *Eco*RI restriction fragment of clone n.13.2 cDNA (containing the entire open reading frame). The carboxyl-terminal region of the deduced amino acid sequence of the cGI PDE contains the putative catalytic domain conserved among mammalian PDE families. A partial cDNA clone, n.2, encoding a truncated, 54-kDa cGI PDE containing the conserved domain was expressed as a catalytically active fusion protein in *Escherichia coli*. cAMP hydrolytic activity was inhibited by cGMP and OPC 3911 but not by rolipram. Thus, this report provides direct proof that the conserved domain contains the catalytic core of cGI PDEs.**

Cyclic nucleotide phosphodiesterases (PDEs) comprise a complex group of enzymes. Five major PDE families or classes with distinctive properties have been identified (1). Analysis of directly determined amino acid sequences of purified PDEs or deduced sequences of cloned PDE cDNAs indicates that four of the five PDE families are products of distinct but related genes and contain a conserved putative catalytic domain of ≈250 amino acids (2). No amino acid sequence has been reported for the so-called cGMP-inhibited low K_m cAMP phosphodiesterase (cGI PDE) family. This PDE family is characterized by its high affinity for cAMP and cGMP (with V_{max} greater for cAMP than for cGMP) and competitive inhibition of its cAMP hydrolytic activity by cGMP and certain positive inotropic agents (3). cGI PDEs with similar properties have been isolated from adipose and cardiac tissues, rat liver, bovine aortic smooth muscle, and human platelets (3). Some cGI PDEs (3–5), including platelet (6) and myocardial (7) cGI PDEs, are apparently regulated by phosphorylation. Incubation of rat adipocytes with insulin, for example, results in phosphorylation and activation of a membrane-associated cGI PDE; this activation is important in the antilipolytic action of insulin (3–5).

Specific inhibition of myocardial cGI PDEs is a primary mechanism of action for a number of positive inotropic agents, including milrinone, amrinone, enoximone, and imazodan (1, 3, 8–13). Inhibition of platelet and vascular smooth muscle cGI PDEs by these drugs is important in their induction of vascular smooth muscle relaxation and inhibition of platelet aggregation (11, 12).

We have characterized cGI PDEs in human platelets and in human myocardial microsomal fractions enriched in sarcoplasmic reticulum (SR). Studies of the steady-state kinetics and inhibitor sensitivity of human SR-associated cAMP PDE activity revealed it to be predominantly cGI PDE (13). K_m values for cAMP and sensitivity to inhibition by cGMP, cilostamide (OPC 3986), OPC 3911, and rolipram were similar for the SR (13) and purified platelet (ref. 3; E.D., M.M., A. Rascón, V. Vasta, E.M., S. Lindgren, K.-E. Andersson, P.B., and V.M., unpublished work) cGI PDEs; anti-platelet cGI PDE antibodies cross-reacted with and immunoprecipitated SR cGI PDE (data not shown). We therefore used oligonucleotide probes based on the partial amino acid sequence of purified platelet cGI PDE to clone a cGI PDE cDNA¶ from a commercially available (Stratagene) human heart cDNA library in λ Zap II. The cardiac cGI PDE cDNA encodes a protein of ≈125 kDa that contains in its carboxyl-terminal region the domain conserved among four other PDE families (2). A truncated, ≈54-kDa form of the cardiac cGI PDE, containing the conserved domain, was expressed as an *Escherichia coli* fusion protein. The cAMP hydrolytic activity of the fusion protein was inhibited by OPC 3911 and cGMP but not rolipram. These results provide direct evidence that the conserved carboxyl-terminal domain contains the cGI PDE catalytic core.

METHODS

Amino Acid Sequences of Peptides from Purified Platelet cGI PDE. Platelets were prepared from outdated platelet-rich human plasma obtained from the National Institutes of Health Clinical Center Blood Bank (Bethesda, MD). cGI PDE was purified from platelet supernatant by chromatography on DEAE-Sephacel and CIT-agarose (an isothiocyanate derivative of cilostamide coupled to aminoethylagarose) by a modification of the procedure developed to purify bovine adipose tissue cGI PDE (14). The purified cGI PDE exhibited two predominant protein-staining bands on SDS/PAGE (62 kDa and a 53/55-kDa doublet). Polyclonal antibodies against purified cGI PDE were produced in rabbits; IgG was purified by chromatography on protein A-Sepharose (Pharmacia) (data not shown).

Purified platelet cGI PDE was partially digested by incubation with several proteases, including trypsin and endoproteinases Glu-C, Lys-C, or Asp-N; peptides were separated by HPLC using Vydac C_{18} or 214TP52 columns with a trifluoroacetic acid/acetonitrile gradient and sequenced on an Applied Biosystems model 477A sequencer with an on-line model 120A phenylthiohydantoin analyzer (data not shown).

cDNA Library Screening. Mixed oligodeoxynucleotide probes (P5 and P8) based on partial amino acid sequences of two platelet peptides, PDE P5 (EFMNYF) and PDE P8

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Abbreviations: cGI PDE, cGMP-inhibited cAMP phosphodiesterase; GST, glutathione *S*-transferase; IPTG, isopropyl β-D-thiogalactopyranoside; SR, sarcoplasmic reticulum.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M91667).

(MMFLDK), with 48 and 16 combinations, respectively, were synthesized using a model 380B DNA synthesizer (Applied Biosystems). A long oligonucleotide (P1A) based on the sequence DHPGRTNAFLVATSAPQAVLYNDR (PDE P1A) was synthesized by using predictions for the most frequent human codons (15). Oligonucleotides were labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP, purified by chromatography on DEAE-Sephacel (Pharmacia), and used to screen $\approx 10^6$ plaques of a human heart λ ZAP II cDNA library (Stratagene) by plaque hybridization. Replicate filters were washed in 5× SSC (1× SSC is 0.15 M NaCl/0.15 M sodium citrate, pH 7) and incubated for 6–7 hr at 37°C (P5, P8) or 65°C (P1A) in prehybridization medium [5× SSC/10× Denhardt's solution (1× is 0.02% Ficoll 400/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone)/0.5% SDS/10 mM Tris, pH 7.5, with denatured salmon sperm DNA at 200 μg/ml].

Hybridizations were carried out at 37°C (P5), 42°C (P8), or 65°C (P1A) in 5× SSC/10× Denhardt's solution/10% dextran sulfate/10 mM Tris, pH 7.5/0.1% SDS containing denatured salmon sperm DNA at 100 μg/ml and the labeled oligonucleotide probes ($\approx 0.5 \times 10^6$ cpm/ng) at 80 ng/ml. Filters were washed twice in 5× SSC/0.5% SDS for 30 min at room temperature and then in 1× SSC for 30 min at 39°C (P5) or 49°C (P8) or for 1.5 hr at 70°C (P1A) before exposure to Kodak XAR film at −70°C with an intensifying screen.

Positive colonies were purified through four successive screenings and hybridizations. Phagemids containing cDNA inserts were excised *in vivo* in the presence of *E. coli* XL1-Blue cells and R408 helper phage. Plasmid DNA was purified by using a Qiagen column according to the manufacturer's directions (Qiagen, Chatsworth, CA).

The nucleotide sequences of cGI PDE cDNA clones were determined by the dideoxynucleotide method using [α-[^{35}S]thio]dATP with T3, T7 and nested internal primers and Sequenase version 2.0 (United States Biochemical).

Northern Blot Analysis. Total RNA was extracted from human heart, human liver, T84 human colon carcinoma cells, human neuroblastoma cells, and HL-60 human promyelocytic cells by the guanidine thiocyanate/phenol extraction technique (16). An ≈4-kilobase (kb) *Eco*RI restriction fragment of clone n.13.2 containing the open reading frame (see Fig. 1) was labeled with [α-^{32}P]dATP by the random priming technique (Stratagene) and used to probe the Northern blots. Blots were washed under high-stringency conditions.

Expression of cGI PDE cDNA Clone n.2 in *E. coli*: Purification and Characterization of a Glutathione *S*-Transferase (GST) Fusion Protein. Clone n.2, encoding much of carboxyl-terminal region (≈54 kDa) of the cGI PDE, was inserted via the *Eco*RI cloning site into pGEX1, a GST fusion-protein expression vector (17). *E. coli* DH5α cells were transformed with expression constructs pGEX1X-2 and pGEX1X-13.2 and streaked on LB/ampicillin (50 μg/ml) plates. After colony hybridization, single positive colonies were picked and grown overnight in LB/ampicillin (50 μg/ml) medium.

Cultures of *E. coli* transformed with plasmids containing antisense or sense inserts were grown overnight and added to 70 ml of LB/ampicillin medium (final OD_{600}, 0.7). The cultures were then divided and isopropyl β-D-thiogalactopyranoside (IPTG, 1 mM final concentration) was added to half. Incubations were continued at 37°C for 4 hr, at which time cell growth was monitored by measuring OD_{600}. Cells (10^9 per ml) were harvested by centrifugation at 14,000 × *g* for 10 sec. Cell pellets were dissolved in 70 μl of 125 mM Tris/10% glycerol/1% SDS/10% 2-mercaptoethanol/0.005% bromophenol blue and subjected to SDS/PAGE.

For purification of the fusion protein and measurement of PDE activity, cell suspensions (50 ml; OD_{600}, 1.3) were centrifuged at 4000 × *g* for 5 min. Cell pellets were resuspended in 5 ml of 20 mM Tris·HCl, pH 7.45/250 mM sucrose/3 mM benzamidine/1 mM EDTA/0.1 mM EGTA/1 mM phenylmethylsulfonyl fluoride containing leupeptin (10 μg/ml) and pepstatin A (10 μg/ml). After sonication (three times for 20 sec), lysates were centrifuged at 14,000 × *g* for 1 min to remove insoluble material. Samples of the supernatants were taken for assay of PDE activity or were mixed with 1 ml of a 50% slurry of glutathione-Sepharose beads and gently agitated for 15 min at room temperature. Beads were collected by centrifugation and washed three times with ice-cold phosphate-buffered saline. The GST fusion protein was eluted by suspending the beads for 15 min at room temperature in 200 μl of 50 mM Tris·HCl, pH 8/10 mM reduced glutathione and centrifuging, a total of three times. Samples of eluates were used for SDS/PAGE or for measurement of PDE activity.

Solubilized cell pellets and purified fusion protein were subjected to SDS/PAGE (10% gel, 45 min, 100 V). The gel was divided; half was stained with Coomassie blue, and the proteins in the other half were transferred to a poly(vinylidene difluoride) (PVDF) membrane (45 min, 50 V, room temperature) in 10 mM Caps, pH 10/10% methanol buffer. After blotting, the PVDF membrane was incubated overnight in blocking solution [bovine serum albumin in 1× Tris-buffered saline (20 mM Tris·HCl, pH 7.5/500 mM NaCl)], washed three times for 20 min in 1× TBS/0.05% Tween 20, and incubated for 4 hr at 4°C with anti-platelet cGI PDE IgG, affinity-purified by a modification (18) of the method of Olmstead. The membrane was then washed three times with 1× TBS/0.05% Tween 20 for 20 min; immunoreactivity was detected (5) with anti-rabbit IgG-linked alkaline phosphatase (Promega).

RESULTS AND DISCUSSION

Oligonucleotide probes were constructed based on the partial amino acid sequences of three platelet peptides and used to screen a human heart cDNA library in λ Zap II. Four of the positive cDNA clones (n.2, n.10, n.12.3, n.13.2) were purified and sequenced. Clone n.2 contained an insert of 1.5 kb; clone n.12.3, a 0.9-kb insert; and clone n.10, a 1.6 kb insert. Clone n.13.2 contained a much larger insert, 7.7 kb. Following digestion of clone n.13.2 with *Eco*RI, 1% agarose gel electrophoresis separated fragments of 4, 1.5, 0.9, 0.8, and 0.5 kb. The 4-kb fragment hybridized with the three oligonucleotide probes and contained nucleotide sequences that included the cDNAs from clones n.2, n.12.3, and n.10. The 4-kb fragment contained an open reading frame of 3423 bases (Fig. 1). The initial ATG is a likely translation initiation site, with putative consensus upstream sequences (19) and secondary structure at the start site (20). In the 4-kb *Eco*RI fragment, the TGA stop codon (nucleotides 3424–3426) is followed by 541 bases of 3′ untranslated region containing a consensus polyadenylylation signal (AATAAA, nucleotides 3825–3830) before the *Eco*RI restriction site (Fig. 1). Polyadenylylation signal sequences, but no poly(A)$^+$ tail, were also found in the 3′-terminal 1.5-kb fragment of clone n.13.2 (data not shown).

On Northern blots the 4-kb cDNA fragment hybridized with an RNA species of 8.3 kb in total RNA from human cardiac ventricle and with RNA species of 7.3 and 4.5 kb from T84 human colon carcinoma cells. The 4-kb cDNA fragment did not hybridize with total RNA from human liver or from human neuroblastoma or HL-60 promyelocytic cells (data not shown).

The open reading frame of 3423 bases predicts a protein of 1141 amino acids with molecular mass of ≈125 kDa, similar in size to the 130-kDa human cardiac SR cGI PDE species detected on immunoblots after partial purification of the SR cGI PDE (data not shown). The deduced amino acid sequence of the carboxyl-terminal region of the cardiac cGI PDE corresponded closely to partial peptide sequences de-

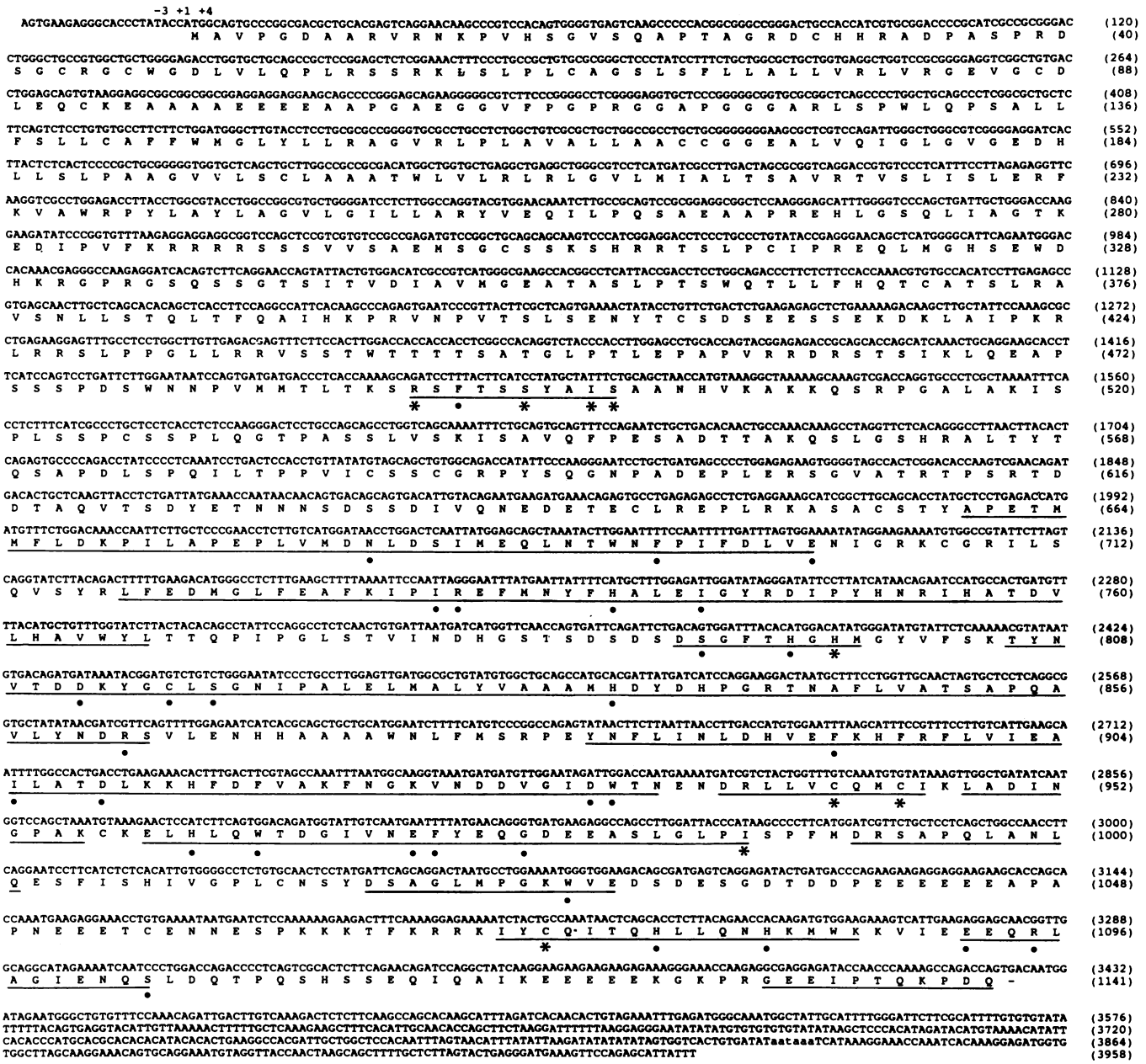

FIG. 1. Nucleotide and deduced amino acid sequences of the 4-kb *Eco*RI fragment of human cardiac cGI PDE cDNA clone n.13.2. Nucleotide residues are numbered from +1 at the first base of the putative initiation codon. Amino acid sequences of overlapping platelet cGI PDE peptides that are identical to the deduced sequence are underlined; asterisks indicate differences between the deduced cardiac cGI PDE sequence and that of platelet cGI PDE; dots indicate ambiguity or lack of identification of platelet residues.

termined from proteolytic digests of pure platelet cGI PDE (Fig. 1).

cGI PDEs are present in both cytosolic and microsomal fractions of homogenates from various cells and tissues (3). The human myocardial enzyme is located in both cytosolic and microsomal fractions (21, 22), whereas the human platelet enzyme is cytosolic (23). The relationship, if any, between cytosolic and membrane-associated cGI PDEs is unknown. Computer analysis of the deduced cardiac cGI PDE sequence with the program SOAP (24) predicts a protein with mixed secondary structure and hydropathy characteristics. Although there is no obvious leader sequence for a hydrophobic region in the amino-terminal region, six putative membrane-association segments are located in the amino-terminal region (amino acids 60–82, 135–153, 157–178, 188–210, 211–229, and 239–255). A hydrophilic domain (amino acids 1020–1070) is found in the carboxyl-terminal region. Given the association of cGI PDE with the SR, and the similarity of sequences in the carboxyl-terminal regions in the platelet and cardiac cGI PDEs, a comparison of amino acid sequences in the amino-terminal domains of platelet and cardiac cGI PDEs may yield important information concerning their intracellular location. Whether potential N-linked glycosylation sites (amino acids 1057 and 1101) in the carboxyl-terminal portion of the cardiac cGI PDE play a role in its association with the SR (or serve another function) is not known.

Analysis of protein sequence data bases (Protein Identification Resource/Swiss-Prot/GenPept-New data base) by the GENEINFO program indicated sequence similarities between cGI PDE and other PDEs. As might be expected from other studies (2), alignment of protein sequences predicted by cGI PDE cDNA with actual and predicted sequences of other PDEs revealed that cGI PDE shares homology with other PDEs in a conserved domain of ≈250 amino acids (Fig. 2).

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cGI     KIPIREFMNYFHALEIGYR--DIPYHNRIHATDVLHAVWYLTTQPIPGLSTVINDHGSTSDSDSDSGFTHGHMGYVFSKT   (806)
CaM     KIPVSCLIAFAEALEVGYSKYKNPYHNLIHAADVTQTVHYIMLHTG-------------------------------   (238)
HPDE1   RIPVDTMVTYMLTLEDHYHA-DVAYHNSLHAADVLQSTHVLLATPA-------------------------------   (253)
cGS     KIDCPTLARFCLMVKKGYR--DPPYHNWMHAFSVSHFCYLLYKNLE-------------------------------   (656)
DRO     MIPPKTFLNFMSTLEDHYVK-DNPFHNSLHAADVTQSTNVLLNTPA-------------------------------   (45)
CON     KVPVEVLTRWMYTVRKGYRAVT--YHNWRHGFNVGQTMFTLLMTGR-------------------------------   (578)
ROS     HIPQEALVRFMYSLSKGYRRIT--YHNWRHGFNVGQTMFSLLVTGK-------------------------------   (578)
          ..          . .*     ..** *. * .    .

cGI     YNVTDDKYGCLSGNIPALELMALYVAAAMHDYDHPGRTNAFLVATSAPQAVLYNDR-SVLENHHAAAAWNLFMSRPEYNF   (885)
CaM     ----------IMHWLTELEILAMVFAAAIHDYEHTGTTNNFHIQTRSDVAILYNDR-SVLENHHVSAAYRL-MQEEEMNV   (306)
HPDE1   ----------LDAVFTDLEILAALFAAAIHDVDHPGVSNQFLINTNSELALMYNDE-SVLENHHLAVGFKL-LQEYNCDI   (321)
cGS     ----------LTNYLEDMEIFALFISCMCHDLDHRGTNNSFQVASKSVLAALYSSEGSVMERHHFAQAIAI-LNTHGCNI   (725)
DRO     ----------LEGVFTPLEVGGALFAACIHDVDHPGLTNQFLVNSSSELALMYNDE-SVLENHHLAVAFKL-LQNQGCDI   (113)
CON     ----------LKKYYTDLEAFAMLAAAFCHDIDHRGTNNLYQMKSTSPLARLHGS--SILERHHLEYSKTL-LQDESLNI   (645)
ROS     ----------LKRYFTDLEALAMVTAAFCHDIDHRGTNNLYQMKSQNPLAKLHGS--SILERHHLEFGKTL-LRDESLNI   (645)
                      .     .* .  .. ** .* *  * .   .   *  . .. *..*.**      . .     .

cGI     LINLDHVEFKHFRFLVIEAILATDLKKHFDFVAKFNGKVNDDVGI---------DWTNENDRLLVCQMCIKLADINGPAK   (956)
CaM     LINLSKDDWRDLRNLVIEMVLSTDMSGHFQQIKNIRNSLQQPEGL--------------DKAKTMSLILHAADISHPAK   (371)
HPDE1   FQNLSKRQRQSLRKMVIDMVLATDMSKHMTLLADLKTMVETKKVTSSGV----LLLDNYSDRIQVLRNMVHCADLSNPTK   (397)
cGS     FDHFSRKDYQRMLDLMRDIILATDLAHHLRIFKDLQKMAEVG----------YDRTNKQHHSLLLCLLMTSCDLSDQTK   (794)
DRO     FCNMQKKQRQTLRKMVIDIVLSTDMSKHMSLLADLKTMVETKKVAGSGV----LLLDNYTDRIQVLENLVHCADLSNPTK   (189)
CON     FQNLNKPQYETVIHLFEVAIIATDLALYFKKRTMFQKIVDACEKMETEEEAIKYVTIDPTKKEIIMAMMMTACDLSAITK   (725)
ROS     FQNLNRRQHEHAIHMMDIAIIATDLALYCKKRTMFQKIVDQSKPYETQQEWTQYMMLDQTRKEIVMAMMMTACDLSAITK   (725)
        . .          .   ...**.  .                          .  .           .*..  .*

cGI     CKELHLQWTDGIVNEFYEQGDEEASL-GLPISPFMDRSAPQ-LANLQESFISHIVGP                          (1011)
CaM     SWKLHHRWTMALMEEFFLQGDHEAEL-GLPFSPLCDRKSTM-VAQSQIGFIDFIVEP                          (426)
HPDE1   PLELYRQWTDRIMAEFFQQGDRERER-GMEISPMCDKHTAS-VEKSQVGFIDYIVHP                          (452)
cGS     GWKTTRKIAELIYKEFFSQGDLEKAM-GNRPMEMMDREKAY-IPELQISFMEHIAMP                          (849)
DRO     PLPLYKRWVALLMEEFFLQGDKERES-GMDISPMCDRHNAT-IEKSQVGFIDYIVHP                          (244)
CON     PWEVQSQVALLVANEFWEQGDLERTVLQQQPIPMMDRNKKDELPKLQVGFIDFVCTF                          (782)
ROS     PWEVQSKVALLVAAEFWEQGDLERTVLQQNPIPMMDRNKADELPKLQVGFIDFVCTF                          (782)
                      ** *** *    .   . .*.       * .*.  .
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FIG. 2. Alignment of the conserved domains of the deduced amino acid sequence of human cardiac cGI PDE and several other PDE isoenzymes: cGI (human cardiac cGI PDE, residues 729–1011), CaM (61-kDa calmodulin-dependent PDE, residues 193–426; ref. 25), HPDE1 (human rolipram-sensitive low-K_m cAMP PDE, residues 209–452; ref. 26), cGS (bovine cardiac cGMP-stimulated PDE, residues 612–849; ref. 27), DRO (*Drosophila* dunce cAMP PDE, residues 1–244; ref. 28), ROS (rod α-subunit cGMP PDE, residues 533–782; ref. 29), and CON (cone α-subunit cGMP PDE, residues 533–782; ref. 30). An initial alignment was produced by using the program CLUSTAL for alignment of multiple sequences (31) and manually adjusted further to align the conserved residues. The perfectly conserved residues are indicated by stars, and partially conserved residues, by periods. Gaps to maximize homology in the alignments are indicated by hyphens.

The conserved region, corresponding to amino acids 736–1011 in the cardiac cGI PDE, is 20–38% identical to the corresponding regions of six other PDEs (25–30). In the conserved region of cGI PDE, however, there is an insertion of 44 amino acids (773–816) apparently completely unrelated to sequences in other PDE families (Fig. 2). A different insertion of 44 amino acids has been found in the same region of the conserved domain of a putative cGI PDE cloned from a rat adipose tissue cDNA library (data not shown). The conserved domains in cGMP-stimulated and calmodulin-sensitive PDEs are more similar to that of cGI PDE than to those of other PDEs (Fig. 2). The cGI PDE sequence differs substantially from other PDE families at the amino and carboxyl ends of the protein.

Since the conserved regions of PDEs are postulated to contain catalytic domains (2), the partial cGI PDE cDNA clone n.2 (1.5 kb), that encodes a truncated 54-kDa protein (amino acids 613–1108) encompassing all of this conserved domain (Figs. 1 and 2), was expressed in *E. coli* (Fig. 3). The GST–cGI PDE fusion protein exhibited the predicted size (81 kDa), cross-reacted with affinity-purified anti-platelet cGI PDE antibody (Fig. 3), and possessed catalytic activity characteristic of cGI PDE (Table 1). cAMP PDE activity was increased in *E. coli* transformed with clone n.2 relative to *E. coli* transformed with clone n.2 in the antisense direction or with the vector alone. As expected for a cGI PDE, PDE activity was inhibited by OPC 3911 and cGMP, but not by 10 μM (Table 1) or 30 μM (unpublished data) rolipram. When compared with purified platelet cGI PDE, the fusion protein was less sensitive to OPC 3911 (IC_{50} 0.4–1.0 vs. 0.03–0.10 μM) and to cGMP (IC_{50} 0.4–1 vs. 0.1–0.3 μM) at 0.5 μM cAMP substrate (data not shown). These apparent differences in pharmacologic sensitivity between the fusion protein and human platelet and SR-associated (13) cGI PDEs may be related to structural constraints inherent in the fusion protein, absence of regulatory regions in the expressed truncated cGI PDE, altered catalytic properties of the fusion protein or truncated cGI PDE, or absence of appropriate posttranslational modification in the prokaryotic expression system.

Expression of the 54-kDa fragment with catalytic activity and sensitivity to specific inhibitors provides additional evidence that the domain conserved among the various PDE families does contain the catalytic core of these enzymes. These findings also complement other studies which indicate that proteolysis of purified bovine brain calmodulin-sensitive (32) and bovine cardiac cGMP-stimulated (33) PDEs with

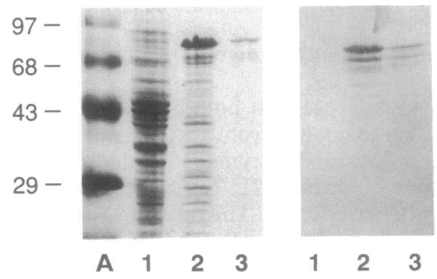

FIG. 3. Western blot analysis of GST–54-kDa cGI PDE fusion protein. Clone n.2 inserted in sense and antisense directions in the pGEX1 vector was expressed as a GST fusion protein. After electrophoresis, proteins were detected by Coomassie stain (*Left*) or Western blot (*Right*). Lane A, standard proteins (kDa at left); lanes 1 and 2, solubilized cell pellets from *E. coli* transformed with antisense or sense pGEX1X-2, respectively, and induced with IPTG; lane 3, purified fusion protein ($\approx$1 μg).

Table 1. Expression of cGI PDE activity in *E. coli*

	− IPTG, −	+ IPTG			
		−	OPC 3911	cGMP	Rolipram
	cAMP hydrolysis, pmol/min per 10^9 cells				
E. coli	1.9	1.7[a]	1.6	2.4	ND
pGEX1X-2	1.6	0.75[a]	1.2	1.0	ND
antisense	ND	0.96[b]	0.86	0.89	1.0
pGEX1X-2	1.2	14.5[a]	4.7	3.1	ND
sense	1.6	15.6[c]	8.7	8.8	16.1
	cAMP hydrolysis, pmol/min per mg of protein				
Fusion	ND	76.6[a]	24.3	25.6	ND
protein	ND	95.0[b]	48.8	47.5	114.4

Cells transformed with pGEX1 (plasmid alone) or pGEX1X-2 (antisense) or pGEX1X-2 (sense) vectors were grown overnight, diluted to OD_{600} 0.7, and incubated for 4 hr without or with 1 mM IPTG. PDE assays (with 0.5 μM [^{3}H]cAMP) were performed in duplicate as described (4) in the presence or absence (−) of inhibitors (1 μM OPC 3911, 1 μM cGMP, or 10 μM rolipram) with total lysate supernatant (*E. coli*, pGEX1X-2 antisense or sense) or with fusion protein (pGEX1X-2 sense) purified by adsorption and elution from glutathione-agarose. ND, not determined.
[a,b,c]The reported data are the results of three separate experiments.

chymotrypsin or trypsin results in the production of catalytically active fragments of 41 kDa and 36 kDa, respectively.

Availability of wild-type and mutant recombinant cGI PDEs should provide valuable information as to structure and function of the several domains involved in catalysis, membrane association, regulation by phosphorylation, and inhibitor recognition. Expression of tissue-specific isoforms might facilitate development of therapeutically useful inhibitors with selectivity for subgroups of the cGI PDE family.

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