Human Platelet cGMP-PDE: Expression in Yeast and Localization of the Catalytic Domain by Deletion Mutagenesis

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Cyclic adenosine monophosphate (cAMP) is an important modulator of platelet responses to agonists. Cyclic nucleotide phosphodiesterase (PDE) controls intracellular cAMP concentrations by hydrolyzing it to AMP. The major PDE activity in platelets is PDE3A (cyclic guanosine monophosphate [cGMP]-inhibited PDE). To obtain structural information on platelet PDE3A, we cloned the enzyme cDNA from a human erythroleukemia cell (HEL) library since the cell line expresses many platelet proteins. This clone consists of 87% of the full-length human myocardial PDE3A cDNA, spanning from nucleotides 456 to 4606, and is identical in sequence. The nucleotide coding for the N terminal 179 amino acid sequence (nt 1-536) as well as four other cDNAs (nt 1459-1632, nt 1765-1986, nt 2152-2538, and nt 2978-3375) obtained by RT-PCR of platelet RNA are also identical to the myocardial sequences, indicating that the HEL, myocardial, and platelet PDE3As are the same. Northern blot analysis of HEL cell RNA detected two mRNAs of 7.5 and 4.4 kb. Four new deletion mutants are reported. PDE 3A Δ1 and PDE 3A Δ2, encoding amino acids 665 to 1141 and amino acids 679 to 1141, respectively, were expressed in a PDE-deficient yeast. They displayed PDE activities of 172 and 79 pmol/mg/min, respectively. PDE 3A Δ3 and PDE 3A Δ4, encoding amino acids 686 to 1141 and 700 to 1141, had no detectable PDE activity. All mutant proteins were expressed as determined by Western blot analysis. These findings localize the PDE3A catalytic domain to within amino acid residues 679 to 1141. © 1996 by The American Society of Hematology.

Cyclic ADENOSINE monophosphate (cAMP) is one of the second messengers that regulates cell proliferation,1 and cellular responses to hormones and ligands.2 In platelets, an increase in the intracellular level of cAMP is associated with an inhibition of platelet responses to agonists, such as shape change, adhesion, aggregation, and release of granule contents.7 Elevation of cAMP is a consequence of the stimulation of adenylate cyclase, the enzyme responsible for its formation from ATP. Alternatively, inhibition of the enzymes responsible for its destruction will increase the intracellular level of cAMP. The essential enzymes for the catalysis of 3′,5′-cAMP to 5′-AMP are cAMP phosphodiesterases (PDEs).

The known PDEs have been expanded recently into seven gene families according to their substrate and inhibitor specificities, kinetic characteristics, tissue distribution, primary mRNA splicing species, and their responses to effectors.8 Hormones, such as insulin, glucagon, and epinephrine, and autacoids, such as prostaglandins, have been reported to regulate PDE activities in different systems.9,11 Cyclic GMP has been shown to stimulate cAMP hydrolysis by some PDEs mediated by homologous noncatalytic cyclic guanosine monophosphate (cGMP)-binding domains while inhibiting others.10 In addition, all PDEs contain a conserved domain of approximately 250 amino acids, which contains the amino acids essential for catalysis.15

At least four forms of cyclic nucleotide PDE activities have been identified in fractions of platelet extracts, namely calmodulin-dependent PDE (PDE1), cAMP-PDE whose activity is stimulated by low levels of cGMP (PDE2),16 low K_m cAMP-PDE which is inhibited by cGMP (PDE3),17 and cGMP-specific PDE (PDE5).18 The four forms of PDEs were affected differently by various PDE inhibitors.18,23 PDE3 is subdivided into PDE3A for cardiac, placental, and platelet PDE3 and PDE3B for fat cell PDE3.3 PDE3A is the most abundant PDE in platelets. It has a low K_m for cAMP and cGMP, but the K_m was 10-fold lower for cGMP than cAMP, making cGMP a potent competitive inhibitor of cAMP hydrolysis.18 Similar PDE3 activity is also found in myocardial and adipose tissue,17 but whether the platelet enzyme is identical to those from other tissues has been unclear.

The importance of cyclic nucleotide PDEs in regulating platelet responses to stimuli is suggested by the ability of certain PDE inhibitors to inhibit platelet responses by elevating intracellular cAMP.22-25 In turn, an increase in cAMP feeds back to limit the increase by stimulating cAMP-dependent protein kinase, which phosphorylates PDE3 and increases its activity.11,12

Native platelet PDE3A has a molecular weight of 110 kD, but it is sensitive to proteolysis and isolation manipulation. The PDE3A isolated from outdated platelets consists of the C-terminal half of the molecule with a molecular weight of 61 kD, which retains the catalytic activity.26 The human heart PDE3A-encoding cDNA protein, cloned by Meacci et al,17 encodes a protein of 125 kD. The conserved region containing the catalytic domain was identified. A recombinant PDE3A truncated at the N-terminal end (amino acids 613-1108) retains the cAMP hydrolytic activity, which is sensitive to inhibition by cGMP and OPC 3911, but not to rolipram, an inhibitor for PDE4, the cAMP-specific PDE.27 Pillai et al28 recently reported that a deletion mutant of human cardiac PDE3A of 46 kD in size had lost the catalytic activity, and that certain sequences between amino acid 608 and 722 were also required for catalytic activity. The precise limits of the catalytic region are not clear.

In order to design specific inhibitors for platelet PDE3A,
Fig 1. Nucleotide sequence and deduced amino acid sequences of platelet CGI-PDE cDNA clone 44-2M-1. The EcoRI cleaved insert of 44-
2M-1 (nt 456-3839) was subcloned in PGEM vector and sequenced. The rest of the insert (nt 3840-4606) was sequenced after PCR amplification
of phage 44-2M-1. DNA sequence of nt 1-535 was obtained by RT-PCR of HEL cell total RNA. The whole insert is 4139 bases long. (1) The
beginning of the cDNA insert. Five platelet RT-PCR cDNAs were sequenced (underlined). (2) Differences between platelet and heart cGI-PDE
sequences (see text). (TG), nucleotide repeat is double underlined. Conserved domain of yeast is arrowed. (W), nucleotide repeat.
Fig 2. Northern blot analysis of HEL cell mRNA. Lane 1 was hybridized to probe 1. Probe 1 = PCR fragment nucleotides 1417-1560 amplifi-
cd by primers pde 10 and pde 6 (pde 10 = 5'-TACACAGCTCCGAT-
TCTTG-3'; pde 6 = 5'-TGAAATTTTAGCGAGGACC-3'). Lane 2 was
hybridized to probe 2. Probe 2 = PCR fragment amplified by primer pde 1 and pde 5 (nucleotide 2522 to 3421).
adATP (600 Ci/mmol; New England Nuclear, Boston, MA). The method with Sequenase from Dr. M. Poncz, The Children’s Hospital of Philadelphia, PA; (2) a hgtl l HEL oligo-dT-directed size was purified from the agarose band by Qiaex gel extraction kit column (Qiagen, Chatsworth, CA).

CCAATCTGGACGAGCITC-3’).

(US Biochemical, Cleveland, OH) and sequenced by the chain termination method using oligonucleotides derived from the human myocardial PDE cDNA sequence that were homologous to the platelet peptide 

Our packaged library consisted of 2 × 10^7 individual plaque-forming units (pfu) before amplification with 5% nonrecombinants.

Library screening. cDNA probes were synthesized by the PCR method with oligonucleotides derived from the human myocardial PDE cDNA sequence 17 that were homologous to the platelet peptide sequence (pde 1: 5’-AAGGAGATGAGAGCCTT-3’) and pde 5: 5’-TGGTCTGGCTTTTGGGTT-3’). The templates used was the total phage DNA prepared from the hgtl HEL cDNA libraries.

Yeast and bacterial strains. Saccharomyces cerevisiae strain GL62 (MATa leu2 ura3-52 adel his3 trpl lys2-208 pde1::LEU2 pepe2::URA3 pep 4::HIS3, which is deficient in yeast PDE1 and PDE2 and aspartyl protease, was used as the host. Yeast vector p138NB19 was used for expression. cDNA was inserted at multiple cloning sites Xho I and Bgl II. Expression is controlled by the copper-inducible CUP1 promoter. All plasmids were propagated in bacterial strain XL1-Blue MRF’ (Strategene, La Jolla, CA) and GM 2163 when the restriction enzyme BsaB1 was used (New England Biolabs, Beverly, MA).

Yeast growth conditions. The media for yeast growth (yeast extract, peptone, and dextrose, YEPD) and synthetic complete minus tryptophan (SC-Trp) were prepared as previously described.20 Yeast transformations were performed using the lithium acetate method.21

Protein concentration determination. Protein concentrations in cell extracts were determined by the Coomassie Plus Protein assay (Pierce, Rockford, IL). Ten microliters of cell extracts or dilutions was mixed with 200 μL of assay reagent and absorbance was measured at 595 nm by Microplate Reader (Bio-Tek, Winooski, VT). Bovine serum albumin dilutions of 0 to 1 mg/mL were used as standards.

Preparation of cell extracts for PDE activity assay. Recombinant yeast cells were grown in SC-Trp medium to A600nm = 0.6. CuSO4 (final concentration = 150 μmol/L) was added to induce expression. After 4 hours, the cells were harvested, washed once in sterile water, and lysed at 4°C in lysis buffer with 0.45 mm glass beads.
beads in a mini-bead beater for small samples (up to 50 mL cultures) or a bead beater for large samples according to manufacturer's specifications (Biospec, Bartlesville, OK). Lysis buffer consisted of 50 mmol/L Tris-HCl, pH 7.5, 5 mmol/L MgCl2, 10 mmol/L dithiothreitol, 0.1% thio-glycol, 0.1% Tween 80, 1.0 mmol/L aminophenylmethylsulfonyl fluoride (APMSF), 20 μg/mL pepstatin A, 100 μmol/L l-lysyl chloromethyl ketone (TLCK), 2 μmol/L benzanidine, 100 μg/mL bacitracin, and 0.3 μmol/L aprotinin. The beads were filtered off by 202 micron nylon (Spectrum, Los Angeles, CA). The filtrates were centrifuged at 12,000g for 30 minutes at 4°C and used for PDE activity assay and Western blot analysis.

In vitro PDE activity assay. The enzymatic activity was assayed in 100 μL final reaction volume containing 50 mmol/L Tris-HCl, pH 7.5, 20 mmol/L MgCl2, 10 mg/mL BSA, and 1 μmol/L [3H]-cAMP (50,000 cpm/assay) at 24°C for 15 minutes. The reactions were stopped with 0.2 mL of 0.2 mol/L ZnSO4 and 0.2 mL of 0.2 mol/L Ba(OH)2. The samples were vortexed and spun at 12,000g for 3 minutes. The supernatants were added to scintillation cocktail and counted. The labeled product of the reaction, [3H]-S'-AMP, was precipitated by BaSO4, and unreacted [3H]-cAMP in the supernatant was measured.

Immunoblot analyses. Sample buffer and prestained protein markers were obtained from New England BioLabs (Beverly, MA). Ten microfilters of each sample plus 5 μL of sample buffer was loaded onto a 5% stacking to 12% running SDS-PAGE gel. The running buffer was 25 mmol/L Tris-HCl, pH 8.8, glycine, 192 mmol/L, SDS 0.1%. Proteins were transferred from the gel to a polyvinylidene difluoride (PVDF) membrane (Immobilon-P, Millipore, Bedford, MA) by Genie electroblotter (Idea, Minneapolis, MN). After blotting, the PVDF membrane was immersed in 5% milk in 1X TBST solution (50 mmol/L Tris pH 8, 150 mmol/L NaCl, 0.05% Tween-20), washed, and incubated with rabbit anti-platelet PDE3A serum (1:500 dilution) for 1 hour at room temperature. Immunoreactivity was detected with goat anti-rabbit IgG-linked alkaline phosphatase. The signal was detected by the chemiluminescent LumPhos-530 system (Boehringer-Mannheim, Indianapolis, IN).

Subcloning of XhoI-2m-1 insert into pGEM vector. Agt11 recombinant phage 44-2m-1 was prepared by plate culture and the DNA was isolated by Qiagen column (Qiagen, Chatsworth, CA). The PDE3A cDNA insert was excised by EcoRI enzyme and subcloned into the EcoRI-digested pGEM-3Zf(+) vector (Promega, Madison, WI). The entire insert was sequenced as above and the clone was designated as pGEM-44-2m-1.

Construction of deletion mutant PDE3AΔ1 for expression in yeast. The human platelet PDE3A yeast expression plasmid PDE3AΔ1 was constructed as follows: A BamHI-Scal fragment (nt 743-nt 927) was prepared from pGEM-44-2m-1 and subcloned into a Sfi I-digested/Klenow filled/BamHI digested pBluescript II SK+ vector (Strategene, La Jolla, CA) to generate pBSPDE3. A 380-bp PCR fragment was synthesized using a forward primer, pde30, with an Xho I site just upstream of the translation start codon, which starts translation at nt 1993 (5'-CCGCCTCGAGAAATGTTTCTGGAGAACAACATTCTT-3') and a reverse primer, pde31, which has PDE sequences around the unique Nde I site at nt 2393 (5'-CCCATATGGGATGTTAAATCCACTGTC-3'). This PCR fragment was cleaved by Xho I and Nde I and subcloned in the Xho I site of pBSPDE3.1 to generate pBS3031. This truncated insert starts with nt. 1993 of the PDE3A cDNA, including the rest of the coding region and 1180 bases of the 3' untranslated region. This insert was recovered by Not I digestion, followed by Klenow fill-in and Xho I digestion and was ligated into the Xho I/Bgl II (Klenow filled) cloning sites of yeast plasmid p138NB. The PCR portion of PDE3AΔ1 was sequenced to confirm that there was no mutation introduced by the PCR reaction. PDE3AΔ1 was transfected into yeast strain GL62. Yeast transformants were picked, grown up in SC-Trp medium, induced with CuSO4, and cell extracts were assayed for PDE activity and analyzed by SDS-PAGE and Western blotting (as described above).

Results and Discussion

DNA cloning and sequencing. HEL cells were chosen as the source of both platelet PDE3A mRNA and cDNA. HEL cells express a number of platelet proteins, including those from granules and membrane receptors. HEL cDNA libraries have been used successfully for the isolation of several platelet-specific cDNAs, such as platelet factor 4 and connective tissue activating peptide III. A PCR fragment of 500 bp was amplified with total phage cDNA prepared from the cDNA libraries as template and oligonu-
cleotides pde 1 and pde 5 as primers. This PCR fragment was subcloned into pCRScript SK(+) (Strategene, La Jolla, CA) and sequenced. The sequence was identical to the published sequence of the human myocardial PDE3A cDNA. This fragment was used to screen the three Agt11 HEL cell cDNA libraries. A total of 35 positive clones were isolated from 10⁶ clones. Clone 44-2m-1 had the longest insert, which had an EcoRI site at nt 3959. This clone is not a full-length cDNA, as is the human heart PDE3A cDNA. It is missing the first 455 nucleotides at the 5' end. This clone and five other clones begin at or near the nucleotide 456, probably due to a blockage of reverse transcription by a secondary structure at this region. Two inverted repeated sequences were identified between nt 310 and 363 by a computer program (DNAsis 2.0; Hitachi Software, New Bruno, CA).

The EcoRI fragment (3491 bp long) was subcloned into pGEM-3ZF(+) plasmid (Promega) and sequenced. The sequence located downstream of nt 3959 was sequenced, after PCR amplification, with phage 44-2m-1 DNA as template. The cDNA sequence is shown in Fig 1. This clone consists of 2967 nucleotides of the coding region and 1179 nucleotides of the 3'-untranslated region. No poly-A tail has been found, though the polyadenylation signal AATAAA is located at nucleotide 3824 according to the numbering system of Meacci et al.⁴¹ The N-terminal sequence was obtained by RT-PCR using HEL cell and platelet total RNA as templates. The sequence was combined with that of clone 44-2m-1 and is shown in Fig 1. The sequence is identical to the human myocardial PDE3A cDNA sequence except at three locations. First, at nucleotides 121-122 of the heart PDE3A, CT should be read as TC. This correction in the DNA sequence was sent to us by Dr V. Manganiello. Second, at nucleotides 187-190, we found TCCG in PDE3A RT-PCR cDNA and clone 13.2 sent to us from Dr V. Manganiello, while the published heart PDE3A cDNA sequence at this position is CTGC. This substitution will change the codons CTG and CCG to TCC and GCG, and the corresponding amino acid residues from L and P to S and A. (Dr V. Manganiello has confirmed this change [personal communication, August 1995].) Third, at nucleotide 570, we found a C in our HEL cell PDE3A cDNA instead of a T in the heart PDE3A cDNA sequence. This substitution will change the codon GCT to GCC but will not change the encoded amino acid alanine. This alteration could constitute a polymorphic marker for the PDE3A locus. We did not find T→C change at nucleotide 2190, as reported by Kasuya et al.⁴⁵

Several other platelet cDNA, produced by RT-PCR, corresponding to regions of the mRNA, were also sequenced (underlined in Fig 1). They are identical to both the HEL cell and myocardial PDE3A cDNA sequence, suggesting that the platelet, HEL cell and myocardial PDE3A mRNAs are transcribed from the same gene. Degerman et al.⁴⁶ have reported the amino acid sequences of 36 platelet PDE3A peptides, which are almost identical to the amino acid sequences deduced from the PDE3A cDNA. Some differences could be due to sequencing problems. The molecular weights of the platelet and cardiac PDE3A full-length proteins determined by SDS-PAGE/Western blot analyses are 110 kD and 125 kD, respectively.¹⁷ ²⁵ ²⁶ ⁴⁰ Since most of the platelet PDE3A activity is present in the cytosolic fraction,²⁶ while that of the myocardial PDE3A is located in the microsomal fraction,²⁵ it is possible that the platelet 110 kD PDE3A protein is different from the cardiac 125 kD PDE3A protein. Whether the platelet 110 kD PDE3A protein is a truncated form of the cardiac 125 kD PDE3A protein is still unclear, since an attempt to sequence the N-terminal amino acids of the 110 kD PDE3A protein has not been successful due to an N-terminal blockage. Nonetheless, we have shown by RT-PCR that HEL cell and platelet total RNA have the capacity to encode for a full-length PDE3A protein identical to the human cardiac PDE3A protein.

In the 3'-untranslated region, we find a previously undescribed stretch of 22 TG dinucleotide repeats at nucleotide 3984. TG microsatellites have been reported to be useful for gene mapping.⁴⁸ A (CA)n-dinucleotide repeat was located at the 3' end of the β-subunit of the cGMP phosphodiesterase gene, and co-dominant Mendelian inheritance was observed.⁴⁹ The polymorphic state of this PDE3A (TG)₂₂ dinucleotide repeat is not yet known. This repeat may be useful as a marker for evolution and linkage studies for the analysis of PDE3A genes in the future.

Northern blot analyses of HEL cell mRNA. Attempts to analyze PDE3A mRNA in platelets by Northern blot were not successful. Very little oligo-dT enriched platelet mRNA was isolated, about 2 to 5 µg U of blood. No PDE3A hybridization signal was observed. Northern blot analysis of the platelet total RNA revealed two faint smeared hybridization signals (data not shown). Therefore, HEL cell mRNA was used to analyze PDE3A mRNA. Two PCR-amplified cDNAs were labeled with ³²P-dCTP and used as probes to analyze the HEL PDE3A mRNAs. One probe includes 114 bases of the conserved region of PDE3A, and another probe is 529 nucleotides upstream from the conserved region and, thus, has no significant homology to other known PDE sequences. Both probes identified two mRNAs of sizes 4.4 and 7.5 kb, respectively (Fig 2). The relationship of these two mRNAs to each other and to the cloned HEL cell PDE3A cDNA is not clear. Clone 44-2M-1 could possibly be encoded by the 7.5-kb mRNA because it covers 4606 nucleotides of sequence and is still missing the poly-A tail. Meacci et al.⁵⁰ detected a cardiac PDE3A mRNA of 8.3 kb by Northern blot analysis. They found 7.3-kb and 4.5-kb PDE3A mRNAs in human colon carcinoma cell T84. Kasuya et al recently identified a 4.4-kb PDE3A mRNA as the major transcript in placenta.⁵¹ The transcription of this placental 4.4-kb mRNA was shown by RNase protection assay to initiate from exon 3 of the same gene that encodes for the 7.5-kb mRNA. In placenta, sequences specific to the 7.5 kb mRNA were also detected by RNase protection assay, but not by Northern blot analysis. These results can be interpreted to indicate that multiple forms of PDE3A differing in the 5' amino terminal may exist in the same or different tissues. Heterogeneity of mRNA has been observed in the type IV PDE family, where more than 15 splice variants are derived by differential splicing and alternate transcription initiation from four different genes.⁴ The data suggest that full-length PDE3A mRNAs were once present in platelets or megakaryocytes as suggested by the presence of PDE3 sequence nt 1-535 in
the cDNA obtained by RT-PCR. We are currently investigating whether the truncated placental PDE3A mRNA is present in platelets by ribonuclease protection assay.

Expression and mapping of the catalytic domain. A conserved region corresponding to amino acids 736-1011 in the myocardial PDE3A was revealed when the amino acid sequence of seven different phosphodiesterases were aligned together.\(^1\) We have aligned two more PDE sequences, Rat PDE III (which is type IV PDE)\(^8\) and Rat fat cell PDE3B\(^1\) with that reported by Meacci et al\(^1\) by DNAsis 2.0 program (Hitachi, San Bruno, CA). Three amino acids previously identified as perfectly conserved residues are reclassified as partially conserved residues: D (991), Q (1001), and F (1004). There are 26 perfectly conserved residues in the nine different phosphodiesterases. Some of them may be important for the catalytic activity.

The conserved region\(^2\) has been postulated to be the catalytic domain of PDEs. The PDE3A protein isolated from outdated platelets (amino acids 660-1141) is a cleaved product of the whole 110 kD protein, which retains full catalytic activity.\(^26\) Meacci et al\(^1\) have constructed a deletion mutant (amino acids 613 to 1108), which retains the catalytic activity. Pillai et al\(^27\) recently presented five deletion mutants of human myocardial PDE3A, four of which retained catalytic activity. One did not have any activity (amino acids 722-1141). They proposed that the amino acid residues from 608 to 722, upstream of the conserved region, were also required for catalytic activity.

We constructed four partial PDE3A clones and expressed them in yeast system. These clones would express PDE3A peptides of calculated molecular masses 60.4, 58.7, 57.5, and 56.0 kD. In Western blot analysis, a protein of about 60 kD was detected by rabbit anti-platelet PDE3A (lanes 4-7, Fig 3). No protein degradation was observed for these mutants. The relative amounts of PDE3 antigen present in the 10 \(\mu\)g of all extracts loaded were similar in all four samples. Cell extracts from PDE3A\(\Delta\)1 have a PDE activity of 172 pmol/mg total cellular protein/min, while that of PDE3A\(\Delta\)2 has a PDE activity of 79 pmol/mg/min (Fig 4). Crude platelet extract has an activity of 200 to 500 pmol/mg/min. The catalytic activity of the crude extract from PDE3A\(\Delta\)1 and PDE3A\(\Delta\)2 mutants is comparable with that observed in platelet preparations and that of the deletion mutants reported by Pillai et al.\(^27\) The other two constructs, PDE3A\(\Delta\)3, encoding for a protein of amino acid residues 686-1141, and PDE3A\(\Delta\)4, encoding for a protein of amino acid residues 700-1141, did not have any PDE activity, suggesting that some or all of the amino acid residues from 679 to 700 are required for the expression of a fully active catalytic domain. The recombinant PDE3A mutant proteins were purified from PDE3A\(\Delta\)1 and PDE3A\(\Delta\)2 yeast cultures by the method of Grant and Colman\(^29\) using DEAE Sepharose and Blue Dextran columns (Sigma, St Louis, MO). The purified PDE3A\(\Delta\)1 protein has a \(K_m\) of 0.20 ± 0.02 \(\mu\)mol/L, and a \(V_{\text{max}}\) of 952 ± 11 nmol/min/mg. The purified PDE3A\(\Delta\)2 has a similar \(K_m\) of 0.23 ± 0.03 \(\mu\)mol/L, and the \(V_{\text{max}}\) is 550 ± 25 nmol/min/mg. The IC_{50} of cGMP, milrinone, and rolipram for PDE3A\(\Delta\)1 are 0.45, 1.5, and >100 \(\mu\)mol/L, and for PDE3A\(\Delta\)2 are 0.50, 3.0, and >100 \(\mu\)mol/L, respectively.

The Km and IC_{50} are virtually identical to the platelet enzyme purified by the same method (Table 1). Determination of IC_{50} for PDE3A\(\Delta\)1 is shown in Fig 5. The \(V_{\text{max}}\) of PDE3A\(\Delta\)2 is 42% lower than that of PDE3A\(\Delta\)1. The lower \(V_{\text{max}}\) may reflect less efficient catalysis by the mutant enzyme or instability of the protein during purification.

The expression of PDE3A in yeast has facilitated the investigation of the structure and function relationship of the conserved region in the protein. In the conserved region, there are 26 perfectly conserved amino acids, some of which are postulated to be important for catalytic activity\(^32\) including seven histidine residues. Metal cations such as Mg\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), and Co\(^{2+}\) affect the activity of phosphodiesterase. Mg\(^{2+}\) and Mn\(^{2+}\) were found to be potent for activating the enzyme.\(^55\) The mechanism by which these metal cations influence the catalytic activity has not been documented. In the conserved domain, some of the 26 perfectly conserved amino acids display a putative zinc-binding motif of HNXXH- and HDXXH- ~25aa-E.\(^35\) Mutating these conserved residues could elucidate the enzyme binding sites and their roles in the formation of the catalytic domain.

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Human platelet cGI-PDE: expression in yeast and localization of the catalytic domain by deletion mutagenesis

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