Molecular Cloning of a Novel Diacylglycerol Kinase Isozyme with a Pleckstrin Homology Domain and a C-terminal Tail Similar to Those of the EPH Family of Protein-tyrosine Kinases*

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A fourth member of the diacylglycerol kinase (DGK) gene family termed DGKδ was cloned from the human testis cDNA library. The cDNA sequence contains an open reading frame of 3,507 nucleotides encoding a putative DGK protein of 130,066 Da. Interestingly, the new DGK isozyme contains a pleckstrin homology domain in a number of proteins involved in signal transduction. Furthermore, the C-terminal tail of this isozyme is very similar to those of the EPH family of receptor tyrosine kinases. The primary structure of the δ isozyme also has two cysteine-rich zinc finger-like structures (C3 region) and the C-terminal C4 region, both of which have been commonly found in the three isozymes previously cloned (DGKs α, β and γ). However, DGKδ lacks the EF-hand motifs (C2) and contains a long Glu- and Ser-rich insertion (317 residues), which divides the C4 region into two portions. Taken together, these structural features of DGKδ indicate that this isozyme belongs to a DGK subfamily distinct from that consisting of DGKs α, β, and γ. Increased DGK activity without marked preference to arachidonoyl type of diacylglycerol was detected in the particulate fraction of COS-7 cells expressing the transfected DGKδ cDNA. The enzyme activity was independent of phosphatidylinerse, which is a common activator for the previously sequenced DGKs. Northern blot analysis showed that the DGKδ mRNA (∼6.3 kilobases) is most abundant in human skeletal muscle but undetectable in the brain, thymus, and retina. This expression pattern is different from those of the previously cloned DGKs. Our results show that the DGK gene family consists of at least two subfamilies consisting of enzymes with distinct structural characteristics and that each cell type probably expresses its own characteristic repertoire of DGKs whose functions may be regulated through different signal transduction pathways.

Diacyglycerol kinase (DGK, EC 2.7.1.107) phosphorylates diacyglycerol to produce phosphatidic acid (1). DGK is thought to play an important role in the signal transduction linked to phospholipid turnover. The roles of diacylglycerol and phosphatidic acid as lipid second messengers have been attracting much attention. Diacylglycerol is known to be an activator of protein kinase C (2, 3), and phosphatidic acid has been reported in a number of studies to modulate a ras GTPase-activating protein (4), phosphatidylinositol (P1)-4-phosphate kinase (5) and many other important enzymes. Phosphatidic acid is also known to have mitogenic effects in a variety of cells (6). Thus, DGK is thought to be one of the key enzymes involved in the cellular signal transduction (1, 7). For instance, DGK was found to be involved in interleukin-2 production in T-lymphocytes (8) and retinal degeneration of Drosophila melanogaster (9).

Many DGK isozymes have been purified from various animal sources, such as two isozymes from porcine brain and thymus (10, 11), three from human platelets (12), two from rat brain (13), and one from bovine testis (14). These isozymes have been described to differ from each other with respect to molecular masses, enzymological properties, activators, and substrate specificity. It is thus likely that these isozymes are operated under distinct regulatory mechanisms. To date, three mammalian DGK genes, DGKs α (15–17), β (18), and γ (19, 20) have been isolated. The protein products of these genes contain four conserved regions, i.e. the N-terminal region (C1), two sets of Ca2+ binding EF-hand motifs (C2), two cysteine-rich zinc finger-like structures (C3), and the C-terminal C4 regions. These isozymes have been shown to exhibit different tissue- and cell-specific modes of expression. Namely, DGKα is most abundant in thymus (15) and oligodendrocytes of brain (17), and DGKβ is particularly enriched in rat neuron (18). DGKγ, on the other hand, is highly expressed in the human retina (19) and rat cerebellar Purkinje cells (20). Despite different expression patterns, the structural and enzymatic properties of the three cloned DGKs are very similar to each other, and all of them are characteristically expressed in the central nervous system. Thus, it seems quite likely that DGK gene family may possibly include additional hitherto unknown members with distinct structural features. Identification of novel DGK isozymes and determination of the complete DGK repertoire represent an important and necessary step for elucidating the exact physiological role of DGK.

In the present investigation, we cloned a novel DGK isozyme, DGKδ, that apparently belongs to a subfamily distinct from that consisting of DGKs previously cloned. Interestingly, the novel isozyme contains a pleckstrin homology (PH) domain at the N terminus and a C-terminal tail similar to those of EPH.

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The abbreviations used are: DGK, diacylglycerol kinase; PS, phosphatidylycerine; PCR, polymerase chain reaction; bp, base pair(s); MOPS, 3-(N-morpholino)propanesulfonic acid; PH, pleckstrin homology; PI, phosphatidylinositol; RACE, rapid amplification of cDNA ends; NRS, rapid amplification of cDNA ends; MOPS, 3-(N-morpholino)propanesulfonic acid; PH, pleckstrin homology; PI, phosphatidylinositol.
family of protein-tyrosine kinases. Moreover, its expression pattern was markedly different from those of other DGKs.

**EXPERIMENTAL PROCEDURES**

**Materials**—Restriction enzymes, DNA modifying enzymes, and linkers were purchased from Takara Shuzo Co., Toyobo Co. and New England Biolabs. α-[32P]dCTP (5000 Ci/mmol) was purchased from Amersham Corp. γ-[32P]ATP was from ICN Biomedicals. 1,2-Ascorbyl-2-oxoglutarate and 1,2-didecanoylphosphatidylethanolamine were from Sigma. Oligonucleotides were synthesized using an Applied Biosystems 380B DNA synthesizer. All other chemicals were of the highest quality commercially available.

**General manipulation of DNA and RNA** was carried out according to the standard procedures (21).

**Poly(A)+ RNA Preparation**—Total RNAs were extracted by the guanidiniumisothiocyanate method (22) from J urkat and HepG2 cells. RNAs obtained were enriched in poly(A)+ RNA content by oligo(dT)-cellulose column chromatography (Pharmacia Biotech Inc. mRNA purification kit). Poly(A)+ RNAs from human retina, testis, and HL-60 cells were purchased from Clontech.

**Reverse Transcriptase Polymerase Chain Reaction (PCR)—**Synthetic oligonucleotide primers (P1: 5'-GGCTCGAGTATGCTCCTGGA-3' and P2: 5'-GGCTCGAGTCTGCTCAGTGA-3') were derived from the consensus amino acid sequence Tyr-Phe-Ser-(Phe/Ile/Val)-Gly-Val-Asp and Pro-Met-Gln-Pro from DGKs. The primer sequences (containing oligonucleotide extension sites) were added to the 5'-ends of both primers.

**To obtain DGK-related core sequences, reverse transcriptase PCR amplification was carried out using GeneAmp RNA PCR kit (Perkin-Elmer Corp.). In brief, first strand cDNA template was synthesized from 0.1 μg of poly(A)+ RNA prepared from human testis in 20 μl of reverse transcriptase buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM MgCl2, 1 mM each dNTP, 1 unit/μl of reverse transcriptase, and 25 μM random hexamers. The reaction mixture was incubated for 10 min at 25°C and then for 30 min at 42°C. After heat treatment (97°C for 5 min), the mixture was added with 80 μl of Taq polymerase buffer containing 20 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 10 units of Taq polymerase, and 1 nmol each of primers P1 and P2. Amplification reactions were carried out for 40 cycles on a GeneAmp PCR System 2400 (Perkin-Elmer Corp.) under the following conditions: 94°C for 1 min, 52°C for 2 min, and 72°C for 2 min, followed by a final elongation step at 72°C for 7 min. Amplified PCR products were subsequently separated by preparative agarose gel electrophoresis. Bands in the position (~500 bp) predicted by reference to the cDNA sequences of DGKs so far cloned were recovered, digested with XhoI, and subcloned into the XhoI site of pBluescript II SK+ (Stratagene).

**Screening of cDNA Libraries—**Randomly primed and oligo(dt)-primed cDNA libraries were constructed in phage λgt10 (Pharmacia TimeSaver cDNA synthesis kit and Stratagene Gigapack II Gold packaging extract), using poly(A)+ RNA prepared from human testis and HepG2 cells. Approximately 8 × 106 (testis) and 1 × 109 (HepG2 cells) bacterial plaques were screened under the stringent condition of hybridization (21). The PCR amplification product (DGKD core) was labeled with [α-32P]dCTP by random oligonucleotide primer extension (24) and used as a probe (Fig. 1). Two representative overlapping clones DGKD10 and DGKD11 were isolated after the fifth injection. The IgG was purified by a column chromatography on Protein A-Sepharose (Pharmacia Biotech Inc.).
RESULTS

Isolation and Identification of DGKδ cDNA—In order to identify novel genes potentially related to the DGK family, a PCR-based amplification strategy was performed. We designed two degenerate oligonucleotide primers, P1 and P2 (Figs. 1 and 2), that corresponded to the amino acid sequences, Tyr-Phe-Ser-(Phe/Leu/Val)-Gly-Val-Asp and Pro-Met-Gln-(Val/Ile)-Asp-Pro (Fig. 1), which was used as a template. The 5′-anchored PCR employing primers R1 and R2 (Figs. 1 and 2) gave rise to three clones (DGKD11–13) with the open reading frame including the initiation methionine codon. Although we further screened the same libraries with DGKD10 cDNA as a probe, we could not obtain clones covering the 5′-region. We, therefore, carried out the RACE-anchored PCR procedure using poly(A)⁺ RNA isolated from HepG2 cells as a template. The 5′-anchored PCR employing primers R1 and R2 (Figs. 1 and 2) gave rise to three clones (DGKD11–13) with the open reading frame including the initiation methionine codon. Although we further screened the same libraries with DGKD10 cDNA as a probe, we could not obtain clones covering the 5′-region.

The composite DGKδ cDNA sequence obtained from clones DGKD3, DGKD10, and DGKD11 (DGKD103, 6214 bp, Fig. 1) has an open reading frame starting from nucleotide 81 to nucleotide 3590 (Fig. 2). The initiation ATG of the open reading frame was identified as the first ATG sequence following an in-frame termination codon at nucleotide 66 to 68. The open reading frame is flanked by 80- (5′-) and 2624-bp (3′-) noncoding nucleotide sequences. A polyadenylation signal (AATAAAA) is found 22 nucleotides upstream of the poly(A) sequence at the 3′-end of the cDNA.

Analysis of the Deduced Amino Acid Sequence for DGKδ—The DGKδ cDNA encodes a putative protein of 1169 amino acid residues having a calculated M₉ of 130,006 (Fig. 2). The primary structure displays several characteristic features shared by other DGK isozymes cloned so far (Fig. 3A). DGKδ contains a tandem repeat of cysteine-rich sequence (residues His₁²²⁰, Cys₁⁶⁹ and His₁³⁹², Cys₂⁴²), namely, His-X₁₀₋₁₂-Cys-X₂₋₃-Cys-X₁₂₋₁₄-Cys-X₂₋₃-Cys-X₂₋₃-His-X₂₋₃-Cys-X₂₋₃-Cys, which is conserved in all of the different DGK isozymes (Fig. 3, A and B). This motif was also found in protein kinase C (35), n-chimaerin (36), and a vav oncogene product (37). The sequence identities of zinc fingers of DGKδ were 33, 33, and 32%, respectively, when compared with those of porcine DGKα (15), rat DGKβ (18), and human DGKγ (19). Although the relative locations of critical residues like His and Cys are well conserved, the overall identity of amino acid sequences of the two zinc fingers is not particularly high among different DGKs (Fig. 3B).

One of the striking structural features of DGKδ is that there is a long insertion (317 residues) that divides the C4 region into two portions, designated C4-a and C4-b subregions (Fig. 3A). A similar division of the C4 region was also noted previously for Drosophila DGK1. However, both sequences of the C4 subregions are very similar to the corresponding parts of the C4 region of other DGKs, exhibiting 51–55% (C4-a) and 38–44% (C4-b) identities, respectively (Fig. 3, C and D). Since the C4 region is well conserved in all mammalian DGK isozymes and Drosophila homologs, it seems to be the catalytic domain. Indeed, we recently found that a DGKα mutant containing only the C4 region showed phospholipid-dependent DGK activity.²

The insert separating the two C4 subregions displayed no significant sequence similarity to that of Drosophila DGK1 and the effect of this insert on the catalytic activity remains unknown. The insertion contains a Glu- and Ser-rich sequence (residues 416–538). In this case, 15 (12.2%) and 22 (17.9%) out of 123 residues are Glu and Ser residues, respectively. The additional characteristic of the sequence of DGKδ in comparison with other DGKs is that DGKδ has neither the N-terminal C1 nor the EF-hand motifs (C2) (Figs. 2 and 3A).

Interestingly, this novel DGK isozyme contains the PH domain (38–40) (residues 10–100) at the N terminus (Figs. 2 and 3E). The PH domain, comprising about 100 amino acids, was originally detected as an internal repeat in pleckstrin, a 47-kDa protein that is the major substrate of protein kinase C in platelets (41). At present, the PH domains have been found in.

² F. Sakane, M. Kai, I. Wada, S. Imai, and H. Kanoh, manuscript in preparation.
a number of proteins involved in signal transduction (38–40) such as a ras-GTPase activating protein, AKT/RAC protein kinase, γ and δ types of PI-specific phospholipase C, a vav oncogene product and protein kinase C, vav oncogene product and protein kinase C, and protein kinase C. All of the most highly conserved residues (Gly14, Leu16, Leu33, Leu54, Phe71, and Trp93 in DGKδ) are completely conserved also in DGKδ (Fig. 3E). The extents of identity and similarity between the PH domain of DGKδ and the corresponding regions of several proteins such as pleckstrin, AKT1, protein kinase C, and phospholipase Cs, are 17–31% and 35–52%, respectively (Fig. 3E). The extents of identity and similarity between the PH domain of DGKδ and the corresponding regions of several proteins such as pleckstrin, AKT1, protein kinase C, and phospholipase Cs, are 17–31% and 35–52%, respectively (Fig. 3E). The C-terminal tail similar to those of the EPH family of receptor tyrosine kinase is dashed underlined.

Fig. 2. Nucleotide sequence and deduced amino acid sequence of DGKδ. Nucleotides and amino acids are numbered at the right, respectively. An in-frame stop codon in the 5′-untranslated sequence and a putative polyadenylation signal in the 3′-untranslated sequence are dotted underlined, respectively. The positions of the reverse transcriptase PCR primers and the 5′-RACE primers are indicated by underlines below the nucleotide sequences. The PH domain is thick underlined. The zinc finger-like cysteine-rich sequences are gray underlined. In this sequence the conserved cysteine and histidine residues are marked by asterisks. The C4-a and C4-b subregions are indicated by double underlines. The C-terminal tail similar to those of the EPH family of receptor tyrosine kinase is dashed underlined.

Transient Expression of DGKδ in COS-7 Cells—In order to confirm that the translational product of the newly isolated cDNA indeed possesses DGK activity, the cDNA (DGKD104) was subcloned into the pSRE expression vector and transfected into COS-7 cells. In order to obtain a maximum expression level, most of the 5′- and 3′-untranslated sequences were removed (Fig. 1). Moreover, several nucleotide substitutions were introduced into the DGKδ cDNA to maximally fulfill Kozak consensus translation initiation sequence (27). The pSRE vector alone was also transfected into COS-7 cells as a control. As shown in Fig. 4A, the expression of the DGKδ enzyme protein with the expected molecular size (130 kDa) could be confirmed by immunoblot analysis with antibodies raised against the C-terminal portion of the enzyme, whereas no band was detected in the control cells. Approximately 80% of the enzyme protein was found to be recovered in the particulate fraction. Two bands of lesser Mr may be proteolytic product of the parent band. As shown in Fig. 4B, DGK activity measured with the particulate fraction of COS-7 cells expressing the DGKδ cDNA was approximately 2.5-fold greater than that measured in cells transfected with the vector alone, whereas there was no corresponding difference in DGK activity in the soluble fraction. Since most of the membrane-associated DGK activity was released when treated with 1 M NaCl, DGKδ appeared to be loosely bound to the membranes as has been noted for rat DGKβ (18) and human DGKγ (19).

When assayed with different molecular species of diacylglycerol including 1-stearoyl-2-arachidonoylglycerol, 1-stearoyl-2-linoleoylglycerol, 1,2-dioleoylglycerol, and 1,2-didecanoylglycerol as substrate, DGKδ showed no marked specificity with regard to the acyl compositions of diacylglycerol (data not shown). PS is known to be a common activator of DGKα, β, γ,
and other enzymes purified (1). However, PS (18.0 mol%) did not activate DGKδ but was rather inhibitory (Fig. 5). The PH domain of pleckstrin was found to bind to PI-4,5-bisphosphate (49). Although 7.0 mol% of PI-4,5-bisphosphate was added to the assay mixture, no increased activity was detected (Fig. 5).

Ca²⁺, which is an activator of DGKα and β (18, 31), also had no effect on the activity of DGKδ (data not shown).

Northern Blot Analysis—The expression pattern of the corresponding transcripts was analyzed in a variety of human tissues and cell lines (Fig. 6). The size of the DGKδ mRNA is ~6.3 kb, which coincides with the composite length of the cDNAs (6214 bp). A relatively high expression level was found in skeletal muscle. The mRNA could be detected to a lesser extent in testis, colon, peripheral blood leukocytes, and HepG2 cells. On the other hand, the brain, retina, thymus, and Jurkat cells displayed extremely low or undetectable levels of the DGKδ mRNA. This expression profile is quite different from those described for DGKα, β and γ, which are most abundant in thymus, brain, and retina, respectively (15, 18, 19).

**DISCUSSION**

Our work adds a new member, DGKδ, to the growing list of mammalian DGK genes. Although rat DGK-III cDNA has recently been cloned (20), this isozyme shared 88% identical amino acid sequence with human DGKγ (19). We thus believe that the DGK-III should be a rat counterpart of human DGKγ. It is of particular interest to note that DGKδ contains the PH domain at the N terminus and the C-terminal tail very similar to those of the protein-tyrosine kinases of EPH receptor family (Figs. 2 and 3A). Such structural features enabled us to classify...
DGK\(\delta\) into the new DGK subfamily (type II), which is distinct with respect to domain structures from that (type I) consisting of DGKs \(\alpha\), \(\beta\), and \(\gamma\) previously cloned. It should be noted here that DGKs belonging to the type I subfamily possess basically the same domain structures despite their markedly different expression pattern. Moreover, DGK\(\delta\) is highly expressed in the skeletal muscle with a very low expression in the brain (Fig. 6). This expression pattern of DGK\(\delta\) is quite different from those of the type I DGK isozymes, which are typically expressed in the central nervous system. It is interesting to see if the type II DGK subfamily also consists of multiple isozymes with related structural characteristics. We may hypothesize that the DGK gene family probably consists of several different subfamilies, as has been known for protein kinase C (3, 35) and PI-specific phospholipase C (50). The divergence of DGK isozymes may reflect their physiological importance and the need to respond to a variety of signaling pathways operating under distinct regulatory mechanisms.

The distribution of the PH domain has so far been limited to cytoskeletal proteins and other proteins involved in cellular signal transductions, many of which are associated with the cell surface and intracellular membranes (40). This domain is thought to serve as the site of protein-protein interaction, which is crucial to the function of these proteins (40). The presence of the PH domain in DGK\(\delta\) suggests that this enzyme would share similar regulatory mechanisms with the signaling and cytoskeletal proteins. Lefkowitz and co-workers (51) have recently found that the PH domains in a number of proteins such as \(\beta\)-adrenergic receptor kinase and PI-specific phospholipase C \(\gamma\) can bind the \(\beta\gamma\) complexes of heterotrimeric G proteins. On the other hand, the PH domains of pleckstrin (49) and the AKT protein kinase family (52) have been found to bind PI-4,5-bisphosphate and PI-3-phosphate, respectively. Although it is possible that the activity of DGK\(\delta\) may be regulated by binding to regulatory proteins or phospholipids, we could not detect at present the effects of at least PI-4,5-bisphosphate on the DGK\(\delta\) activity.

The EPH family with a dozen members is currently known to be the largest subfamily of receptor protein-tyrosine kinases (53). Oncogenic activities of this receptor family have also been
gene induced by tumor necrosis factor-
been found to be B61, the protein product of an early response
described previously (54). Recently, one of their ligands has
DGK activity was measured as described under "Experimental
sion(s) of this domain remains to be explored. We are currently
vestigating the possibility that the action of DGK δ is regulat-
ed by tyrosine phosphorylation.
DGK δ has a long insertion composed of 317 residues between the
C4-a and C4-b subregions. This insertion occurs only in
DGK δ among the sequenced mammalian DGKs, but a similar
insertion was described previously for Drosophila DGK1. How-
ever, there was no significant sequence homology between the
insertions of DGK δ and the Drosophila homolog. Moreover, the
insertion did not have significant similarities to other se-
quences deposited in protein data base. We noted that the
insertion of DGK δ contains Glu- and Ser-rich sequences. It is
interesting to note that casein kinas I, II and mammary
gland casein kinase phosphorylate Ser residues in the se-
quencies, Glu-X-Ser, Ser-X-Glu, and Ser-X-Glu, respec-
tively (57). DGK δ contains several of these sequences (Fig. 2). It
is therefore possible that the insertion of DGK δ serves as a
phosphorylation site(s) of such protein kinases. The sequences
C4 region were most highly conserved in all DGK isozymes
and the amino acid sequence of the C4 region with other nucletide-
dependent DGK activity. These results indicate that the zinc
finger binding proteins (58) did not reveal candidate ATP-binding
site(s) of DGK. The present work showed that the zinc finger
was previously shown that the zinc fingers of human DGK
δ is incapable of phorbol ester binding (59). Furthermore, we found
that a DGK δ mutant lacking the zinc fingers exhibited a phos-
pholipid-dependent DGK activity.2 These results indicate that
the DGK zinc fingers are not the sites of phorbol ester or DG
binding. The function of this motif is thus entirely unknown for
DGKs. Recently the zinc fingers of protein kinase Cε and Raf-1
kinase have been shown to be a subcellular localization domain

![Fig. 4. Transient expression of DGKδ in COS-7 cells. A. immu-
noblot analysis of translation product of DGKδ cDNA in COS-7 cells.
Particulate (P) and soluble (S) fractions (10 μg of protein each) from
COS-7 cells transfected with pSRE-DGKδ (DGKδ) or the vector alone
(pSRE) were separated by SDS-polyacrylamide gel electrophoresis and
transferred to a nitrocellulose membrane. The DGK
activity was measured as described under "Experimental
Procedures." B, particulate (ppt) and soluble (sup) fractions were sepa-
rated from COS-7 cells transfected with pSRE-DGKδ or pSRE vector
alone. DGK activity was measured as described under "Experimental
Procedures." The results are means ± S.D. of three independent
experiments.](http://www.jbc.org/)

![Fig. 5. Effects of PS and PI-4,5-bisphosphate on the activity of
DGKδ. DGK activity was measured in the presence of 18.0 mol% of PS
or 7.0 mol% of PI-4,5-bisphosphate (PIP2). To facilitate comparison,
background activities (those of the control cells transfected with the
vector alone) were subtracted, and the results were expressed as per-
zent of the value obtained in the absence of activators. The values
are the average of duplicate determination, which differed by less than 10%
of the mean. Similar results were obtained in two repeated
experiments.](http://www.jbc.org/)

![Fig. 6. Northern hybridization analysis of human tissues and
cells. Poly(A)+ RNA (2 μg each) was analyzed as described under
"Experimental Procedures." Except for poly(A)+ RNAs from retina,
HL-60, Jurkat, and HepG2 cells, commercially available multiple tissue
Northern Blot I and II (Clontech) were employed. The filters were
hybridized with the Xho-Nol fragment of DGKD3 labeled with 32P
and exposed for 60 h with intensifying screens. Almost equal amount of
RNA had been loaded, as judged from hybridization of the same filter
with a human β-actin cDNA probe (data not shown).](http://www.jbc.org/)
and an interaction site with p21ras (61, 62), respectively. It is therefore likely that some novel and unexpected functions would be ascribed to the DGK zinc fingers in future studies.

The DGK δ cDNA gave only 2.5-fold increase of DGK activity in the transfected COS-7 cells (Fig. 4B). This contrasts with the high enzyme activities of other DGKs transfected under the same experimental conditions (19). This low level of enzyme activity hindered us from defining detailed enzymological properties of DGK δ. This may be partly due to the low expression of the enzyme protein or to proteolytic degradation of the expressed enzyme as noted in immunoblotting (Fig. 4A). In view of the apparent lack of phosphatidylserine dependence of the DGK δ activity (Fig. 5), the enzyme assay conditions developed for the type 1 enzymes may not be suitable for this isozyme. It is also possible that DGK δ requires unknown activator(s) or its phosphorylation on serine or tyrosine residues to exert full catalytic activity. In this respect, we could not deny the possibility that DGK δ might prefer substrates other than DG. However, we could not detect the enzyme activity when the COS cell extracts were tested with PI, 1- or 2-monoacylglycerol, ceramide, and sphingosine as substrates. The enzymological studies using purified DGK δ are needed to define the regulatory mechanisms of this isozyme.

Despite interesting domain structures, the function of DGKs in cellular phospholipid metabolism is largely unknown. The present work further points to the potential importance of DGKs as signaling molecules by disclosing the existence of a novel isozyme possessing the PH and EPH C-terminal tail domain. The repertoire of DGK isozymes elucidation at the molecular level for the type I enzymes may not be suitable for this isozyme. It is therefore likely that some novel and unexpected functions are needed to define the regulatory mechanisms of DGKs would reveal the physiological significance of these proteins with unique structures.

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