Overexpression of a Mammalian Ethanolamine-specific Kinase Accelerates the CDP-ethanolamine Pathway*

Received for publication, September 26, 2000, and in revised form, October 19, 2000
Published, JBC Papers in Press, October 23, 2000, DOI 10.1074/jbc.M008794200

Ethanolamine kinase (EKI) is the first committed step in phosphatidylethanolamine (PtdEtn) biosynthesis via the CDP-ethanolamine pathway. We identify a human cDNA encoding an ethanolamine-specific kinase EKI1 and the structure of the EKI1 gene located on chromosome 12. EKI1 overexpression in COS-7 cells results in a 170-fold increase in ethanolamine kinase-specific activity and accelerates the rate of [3H]ethanolamine incorporation into PtdEtn as a function of the ethanolamine concentration in the culture medium. Acceleration of the CDP-ethanolamine pathway does not result in elevated cellular PtdEtn levels, but the excessive PtdEtn is degraded to glycerophosphoethanolamine. EKI1 has negligible choline kinase activity in vitro and does not influence phosphatidylcholine biosynthesis. Acceleration of the CDP-ethanolamine pathway also does not change the rate of PtdEtn formation via the decarboxylation of phosphatidylserine. The data demonstrate the existence of separate ethanolamine and choline kinases in mammals and show that ethanolamine kinase can be a rate-controlling step in PtdEtn biosynthesis.

Ethanolamine kinase or EKI† (ATP:ethanolamine O-phosphotransferase, EC 2.7.1.82) catalyzes the first step of PtdEtn biosynthesis via the CDP-Etn pathway. ECT, a cytidylyltransferase, and EPT, an amino alcohol phosphotransferase, catalyze the subsequent two steps, and together these three enzymes constitute the de novo pathway for PtdEtn formation. The decarboxylation of PtdSer is an alternate route for PtdEtn production and is functionally important in cultured cell lines (1, 2), although the CDP-Etn pathway is considered a major route for PtdEtn synthesis in most mammalian tissues (3–6). PtdEtn is an abundant phospholipid in eucaryotic cell membranes, and the ECT reaction is thought to be a major regulator of its synthesis (7). EKI has been proposed as a regulatory step based on theoretical considerations (8). However, experimental investigations result in conflicting conclusions. Experiments performed with rat hepatocytes (4, 9) demonstrate that the supply of ethanolamine limits the rate of PtdEtn production at concentrations below 30 μM. Only at higher concentrations of ethanolamine does accumulation of phosphoethanolamine occur, indicating ECT as the rate-limiting enzyme of the pathway. On the contrary, McMaster and Choy (10) report that the EKI step is rate-limiting with increased ethanolamine concentrations using a hamster heart perfusion model. Both studies agree that the rate of PtdEtn synthesis is dependent on the extracellular ethanolamine concentration (9, 10) and that the stimulation of PtdEtn biosynthesis occurs at physiological levels of 20–50 μM (9).

There is a long standing discussion as to the number and specificity of the enzymes that catalyze the phosphorylation of ethanolamine and choline. In yeast, there are two enzymes that are able to phosphorylate choline and ethanolamine, and these enzymes are annotated based on their preferred substrate specificities. The CKI enzyme has a specific activity 3.6-fold higher with choline compared with ethanolamine. The EKI enzyme has a specificity constant for ethanolamine 2-fold higher than that for choline (11). CKIs have been purified from several mammalian tissues. Preparations from rat kidney (12), liver, and brain (13) exhibit significant ethanolamine kinase activity with computed ratios of CKI to EKI activity of 1.4, 3.7, and 1.3, respectively. A mammalian CKI cDNA clone also encodes an enzyme with dual specificity (14), and additional highly homologous CKI clones have been identified (15, 16). These results suggest that in mammals both activities reside on the same protein.

However, the existence of mammalian ethanolamine-specific kinases cannot be excluded by the data. Ethanolamine-specific kinase activities have been identified (17–20) and partially purified. EKI activities 1 and 2 isolated from rat liver are separable distinct proteins (21). EKI1 is estimated to be 36 kDa and does not exhibit CKI activity (21). EKI2 is estimated to be 160 kDa and prefers ethanolamine but also has significant CKI activity (21). An EKI of 42 kDa has been purified from human liver with a 5-fold preference for ethanolamine compared with choline (19), and also an EKI activity of 86 kDa has been identified from rat liver and HeLa cells using an antibody raised against the Drosophila EKI (20). The discovery of the Drosophila EKI (22) encoded by the eas gene establishes the existence of an ethanolamine-specific kinase in higher eucaryotes. Expression of the eas gene product as a glutathione S-transferase-fusion protein in Escherichia coli reveals a kinase that is highly specific for ethanolamine with negligible CKI activity (20). A defect in the Drosophila eas results in a bang-sensitive paralytic phenotype, although the molecular link between the absence of the ethanolamine kinase activity and the physiological abnormality has not been established (22).

* This work was supported by National Institutes of Health Grant GM 45737 (S. J.), Cancer Center (CORE) Support Grant CA 21765, and the American Lebanese Associated Charities. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡‡ The abbreviations used are: EKI, ethanolamine kinase; Etn, ethanolamine; PtdEtn, phosphatidylethanolamine; PtdCho, phosphatidylcholine; PtdSer, phosphatidylserine; ECT, cytidylyltransferase; CKI, choline kinase; contig, group of overlapping clones; kb, kilobase pair(s) or kilobase; bp, base pair(s).

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In the present study, we identify a human kinase that is highly specific for ethanolamine (EKI1). Moreover, we demonstrate that EKI1 overexpression accelerates PtdEtn biosynthesis as a function of the exogenous ethanolamine, whereas it has no effect on PtdCho formation.

EXPERIMENTAL PROCEDURES

Materials—Sources of supplies were as follows: [3H]-[3H]serine (specific activity 30 Ci/mmol) was from Amersham Pharmacia Biotech; [methyl-3H]-choline (specific activity 80 Ci/mmol) and [1-3H]ethanolamine hydrochloride (specific activity 55 Ci/mmol) were from Sigma, and Rapid Labelled Chemicals (St. Louis, MO). Lipofectamine reagent was from Life Technologies, Inc.; restriction endonucleases and other molecular biology reagents came from Promega; pcDNA3 plasmid came from Invitrogen; cDNA clones AA598956 and R15326 were from American Type Culture Collection; human liver poly(A)RNA was from CLONTECH; ATP was from Roche Molecular Biochemicals; thin layer chromatography plates came from Analytic; choline, ethanolamine, phosphorylcholine, phosphorylethanolamine, CDP-ethanolamine, and glyceroethylphosphorylethanolamine came from Sigma; and phospholipid standards were from Avanti Polar Lipids. All other supplies were reagent grade or better.

Cloning of the EKI cDNA and Construction of the Expression Vector—The water-soluble metabolites from the [3H]choline-labeling experiments was performed by spotting the aqueous phase on a preadsorbed Silica Gel G plate, which was developed in the solvent system in 95% ethanol, 2% ammonium hydroxide, 1:1 (v/v). The Rf values for ethyamine, cholinephosphocholine, glycerophosphocholine, and CDP-choline were 0.06, 0.17, 0.35, and 0.59, respectively.

The analysis of the chloroform-soluble phospholipids from the labeling experiments was performed by spotting the lipid phases on Silica Gel 60 plates and developing in the solvent system CHCl3;CH2OH: NH4OH, 60:35:8 (v/v/v). The Rf values for PtdSer, sphingomyelin, PtdCho, and PtdEtn were 0.22, 0.26, 0.45, and 0.76, respectively.

Choline and Ethanolamine Kinase Assays—COS-7 cell pellets transfected with either plasmid pPJ96, pAL10, or a control plasmid were incubated in lysis buffer (20 mM Tris-HCl, pH 8.0, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 2 μg/ml apro tin) for 1 h on ice. The cells were disrupted by sonication, and the particulate fraction was removed by low speed centrifugation. Ethanolamine or choline kinase activities were determined essentially as described previously (11, 24). The standard assays contained 100 mM Tris-HCl, pH 8.0, 0.1 mM ATP, 0.3 mM [3H]ethanolamine (specific activity, 397 Ci/mmol), or 0.25 mM [3H]choline (specific activity 398 Ci/mmol) in a final volume of 50 μl. The reaction mixtures were incubated at 37 °C for 15 min. The reaction was stopped by the addition of 5 μl of 0.5 M Na3EDTA, and the tubes were vortexed and placed on ice. Next, 35 μl of each sample was spotted on preadsorbed Silica Gel G thin layer plates, which were developed with 2% ammonium hydroxide, 95% ethanol, 5.1 (v/v). Phosphoethanolamine and phosphocholine were identified by comigration with standards that were scraped from the plate and quantitated by liquid scintillation counting. Protein was determined according to the Bradford method (25).

RESULTS

Isolation of a Human EKI1 cDNA—BLAST searches of the public expressed sequence-tagged data base identified a cDNA clone (GenBank™ accession number AA598956) with a sequence similar to the Drosophila gene. The clone was annotated, and the complete cDNA sequence was determined. In-frame stop codons were not identified at the 5′-end of the cDNA; however, the clone was 2040 bp long, and an open reading frame was predicted previously (11, 24). The standard assays contained 100 mM Tris-HCl, pH 8.0, 0.1 mM ATP, 0.3 mM [3H]ethanolamine (specific activity, 397 Ci/mmol), or 0.25 mM [3H]choline (specific activity 398 Ci/mmol) in a final volume of 50 μl. The reaction mixtures were incubated at 37 °C for 15 min. The reaction was stopped by the addition of 5 μl of 0.5 M Na3EDTA, and the tubes were vortexed and placed on ice. Next, 35 μl of each sample was spotted on preadsorbed Silica Gel G thin layer plates, which were developed with 2% ammonium hydroxide, 95% ethanol, 5.1 (v/v). Phosphoethanolamine and phosphocholine were identified by comigration with standards that were scraped from the plate and quantitated by liquid scintillation counting. Protein was determined according to the Bradford method (25).

Northern Blot Analysis—Mammalian Ethanolamine Kinase 2175

Subcellular Fractionation—COS-7 cells transfected with either pAL10 or pPJ96 were incubated in lysis buffer (20 mM Tris-HCl, pH 8.0, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 2 μg/ml aprotinin) for 1 h on ice followed by sonication. Nuclear and mitochondrial fractions were separated by centrifugation at 10,000 × g for 15 min. Microsomes were separated by centrifugation at 100,000 × g for 60 min. The pellets were resuspended in buffer, and the ethanolamine kinase activity was determined as described above.

Proteins were run on 8.5% polyacrylamide gel and transferred to nitrocellulose by Western blotting. The blots were hybridized with [32P]-labeled random primed probes prepared from the 1.1-kb HindIII-Ndel fragment of AA598956 in the case of EKI1, or in the case of EKI2 the blots were hybridized with a probe that was prepared using the 1.5-kb HindIII-Ndel fragment of R15326 as template. Probes were made using [32P]dCTP according to manufacturer's instructions.

RESULTS

Isolation of a Human EKI1 cDNA—BLAST searches of the public expressed sequence-tagged data base identified a cDNA clone (GenBank™ accession number AA598956) with a sequence similar to the Drosophila gene. The clone was annotated, and the complete cDNA sequence was determined. In-frame stop codons were not identified at the 5′-end of the cDNA; however, the clone was 2040 bp long, and an open reading frame was identified between 252 and 1343 bp. Subsequent BLAST searches of the public high throughput genomic sequence data base identified the genomic sequence upstream of the 5′-end of the cDNA clone AA598956. Based on this information we designed primers and performed reverse transcriptase-polymerase chain reaction using human liver mRNA. The 133-bp product contained 103 additional nucleotides at the 5′-end. This additional sequence contained an in-frame methionine and an upstream stop codon. The starting methionine resides in a favorable Kozak translation initiation sequence (26, 27). We call the above product cDNA EKI1. The EKI1 cDNA is predicted to encode a protein of 452 amino acids with a molecular size of 49.7 kDa.
Searches of the data base revealed that expressed sequence-tagged clones are highly similar to the sequence of EKI1. Using five clones (GenBank™ accession numbers AI798887, AI972267, AW271947, AW960911, and U69210), we were able to reconstruct in silico a 2488-bp cDNA with an open reading frame specifying a protein 477 amino acids long, which we call EKI2a (Fig. 1). This open reading frame does not have a stop codon upstream of the postulated starting methionine; therefore, the possibility that EKI2a is longer cannot be excluded. However, the corresponding mouse cDNA predicts a protein that starts from the methionine as shown in Fig. 1 and has an in-frame upstream stop codon verifying that this is the correct initiating methionine in this species (data not shown).

A sequence highly similar to EKI2a was identified in the data base (GenBank™ accession number AK001623), which is a splice variant of EKI2a. They are identical in the first 338 amino acids, but they have a different carboxyl terminus. Currently, there are no biochemical data establishing that EKI2a or EKI2β is an ethanolamine-specific kinase. Fig. 1 shows the similarity of the predicted EKI1 amino acid sequence to the sequences of the EKI2a, Drosophila eas, and yeast EKI1.

BLAST searches of the high throughput genome sequences revealed that the EKI1 gene is located on human chromosome 12. It spans ~60.5 kb, and it consists of 8 exons and 7 introns (Fig. 2). The exon sizes range between 77 and 828 bp. The intron sizes range between 77 and 828 bp. There is a mouse homologue to EKI1 that also localizes to mouse chromosome 12.

The exon sizes range between 0.3 and 18.1 kb. There is a mouse homologue to EKI1 that also localizes to mouse chromosome 12. In contrast, EKI2 as well as its mouse homologue are located on chromosomes 1 in both species. The structure of the EKI2 gene awaits the assembly of the raw genomic sequence data.

***Tissue Distribution of EKI1—***The relative abundance of EKI1 and EKI2 mRNAs in a variety of human tissues was addressed by Northern blot analysis (Fig. 3). The blots were probed with a 32P-labeled fragment from the 5’-end of the EKI1 cDNA. EKI1 is uniformly distributed in the tissues examined (Fig. 3A). A ~2.2-kb transcript was detected in several tissues. This transcript corresponds to the size predicted from the cDNA sequence (Fig. 2). An intense signal of ~1.7 kb was detected in the testis, but it is not clear if it is due to degradation, alternative splicing, or alternative polyadenylation of the mRNA. The band at about 4.5 kb in several of the lanes corresponds to the position of 28 S ribosomal RNA and is probably due to nonspecific binding of the probe to rRNA or alternatively represents incompletely spliced EKI1 mRNA.

EKI2 was selectively expressed in kidney, liver, ovary, testis, and prostate, but the signal was below detectable levels in the other tissues examined (Fig. 3B). Two sizes of EKI2 transcripts were detected that probably correspond to the two splice variants EKI2α and EKI2β as discussed above. We postulate that the larger transcript of ~3 kb corresponds to EKI2α, whereas the smaller transcript of ~2.5 kb corresponds to the β-isofrom of EKI2.

**Expression and Activity of EKI1—***COS-7 cells were transfected with pAL10, pPJ96, or pcDNA3.1 vector control, and 48 h later cell lysates were prepared. The lysates were assayed for ethanolamine kinase and choline kinase activities. Ethanolamine kinase enzymatic activity was significantly higher in cells transfected with pAL10, pPJ96, or pcDNA3.1 vector control, and 48 h later cell lysates were prepared. The lysates were assayed for ethanolamine kinase and choline kinase activities. Ethanolamine kinase enzymatic activity was significantly higher in lysates from both pAL10 and pPJ96 transfected cells (Fig. 4A). Endogenous ethanolamine kinase activity was significantly higher in lysates from both pAL10 and pPJ96 transfected cells (Fig. 4A). Endogenous ethanolamine kinase activity was significantly higher in lysates from both pAL10 and pPJ96 transfected cells (Fig. 4A). Endogenous ethanolamine kinase activity was significantly higher in lysates from both pAL10 and pPJ96 transfected cells (Fig. 4A). Endogenous ethanolamine kinase activity was significantly higher in lysates from both pAL10 and pPJ96 transfected cells (Fig. 4A). Endogenous ethanolamine kinase activity was significantly higher in lysates from both pAL10 and pPJ96 transfected cells (Fig. 4A). Endogenous ethanolamine kinase activity was significantly higher in lysates from both pAL10 and pPJ96 transfected cells (Fig. 4A). Endogenous ethanolamine kinase activity was significantly higher in lysates from both pAL10 and pPJ96 transfected cells (Fig. 4A).
specific activity to 2.62 pmol/min/μg. The ratio of ethanolamine to choline kinase specific activities in lysates overexpressing pAL10 was 108, and in lysates overexpressing pPJ96 the ratio was 35. These data indicated that EKI1 encoded a kinase that was highly selective for ethanolamine.

The amino terminus of EKI1 contains a region rich in hydrophobic residues (amino acids 65–90), suggesting that these might constitute a membrane interaction domain. Two independent predictive algorithms indicated that a transmembrane helix was likely found in amino acids 66–90. To test the possibility that the EKI1 is a membrane-associated protein, we transfected COS-7 cells with either pAL10 or pPJ96, which lacked the predicted transmembrane helix and fractionated the cell extracts. In both cases, 96% of the total ethanolamine kinase activity was recovered in the cytosolic fraction with the remaining activity distributed almost equally between the nuclear plus mitochondrial and microsomal fractions (data not shown). The data indicated that the enzymes specified by the expression of the EKI1 cDNAs were not associated with cell membranes.

**Effect of EKI1 Expression on Phospholipid Metabolism**—To investigate the contribution of the EKI1 step in the regulation of the CDP-Etn pathway, we overexpressed pPJ96 in COS-7 cells, labeled the cells with the appropriate precursor, and monitored the incorporation of the label into the metabolites. All experiments were performed with both the pAL10 and the pPJ96 expression vectors with identical results.

Two sets of COS-7 cells were transfected with either EKI1 expression vector or control vector. After 24 h, cells within a set were pooled and replated to avoid variation in transfection efficiency among replicated dishes. At 48 h after transfection, the medium was changed, and ethanolamine was added at the concentrations indicated in Fig. 5. Cells were radiolabeled with [3H]ethanolamine for 6 h at 37 °C and then harvested. The

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**Fig. 2. Structure of the human EKI1 gene.** Genomic sequence was derived from the data base entry (GenBank™ accession number AC027624). The EKI1 gene spans ~60.5 kb. The length of each exon is as follows: exon 1, 407 bp; exon 2, 261 bp; exon 3, 145 bp; exon 4, 150 bp; exon 5, 87 bp; exon 6, 163 bp; exon 7, 77 bp; and exon 8, 828 bp. The sizes of the introns are indicated on this figure. The gene is located on chromosome 12. Stars indicate stop codons.

**Fig. 3. Distribution of EKI1 and EKI2 in human tissues.** Human tissue Northern blots were hybridized and washed according to the manufacturer’s instructions. A, pattern of EKI1 expression in human tissues. The blots were hybridized with a 32P-labeled probe prepared from the 1.1-kb HindIII-NdeI fragment of EKI1. B, pattern of EKI2 expression in human tissues. The blots were hybridized with a 32P-labeled probe prepared from the 1.1-kb HindIII-NdeI fragment of the expressed sequence-tagged clone R15326.

**Fig. 4. EKI1 is an ethanolamine-specific kinase.** COS-7 cells were transfected with either pAL10 (●), pPJ96 (○), or the control plasmid pcDNA3 (○). After 48 h, the cells were lysed, and the soluble extracts were evaluated for ethanolamine and choline kinase activities. A, ethanolamine kinase assays were performed as described under “Experimental Procedures.” B, choline kinase assays were performed as described under “Experimental Procedures.” The experiment was performed twice with similar results. The data from one experiment are shown.
incorporation of [3H]ethanolamine into PtdEtn increased in correspondence with increasing concentrations of ethanolamine in the medium (more than 99% of the label incorporated into the lipid phase comigrated with a PtdEtn standard). Overexpression of ethanolamine kinase activity did not affect the amount of the label incorporated into PtdEtn when the concentration of ethanolamine in the medium was below 10 μM (Fig. 5). However, at higher concentrations of ethanolamine (16 and 32 μM), EKI1 overexpression resulted in increased radiolabeling of PtdEtn. At 16 μM we observed a 1.5-fold increase (from 647,536 to 924,660 cpm/mg of cell protein), whereas at 32 μM we detected an ~2.5-fold increase in the incorporation of [3H]ethanolamine into PtdEtn (from 539,519 to 1,393,556 cpm/mg of cell protein) (Fig. 5). These data indicated that the phosphorylation of ethanolamine by EKI1 modulated the rate of PtdEtn biosynthesis at physiological concentrations of ethanolamine.

The distribution of [3H]ethanolamine among the water-soluble metabolites revealed that ECT was still the slow step in the CDP-ethanolamine pathway (Fig. 6). At 32 μM ethanolamine, overexpression of EKI1 caused a 20-fold increase in the incorporation of the label into phosphoethanolamine, whereas it did not significantly affect the levels of ethanolamine or CDP-ethanolamine. We also detected a 3-fold increase in the levels of cellular glycerophosphoethanolamine. These data indicated that the overexpression of EKI1 cDNA perturbed PtdEtn metabolism via the CDP-ethanolamine pathway, which caused an accumulation of phosphoethanolamine, and that the additional PtdEtn produced was degraded to glycerophosphoethanolamine.

To examine whether the overexpression of EKI1 cDNA increased the cellular PtdEtn levels, we transfected COS-7 cells with either EKI1 expression vector or a control vector labeled with [32P]orthophosphate for 48 h to steady state and quantified the incorporation of the label into the lipid phase (data not shown). Overexpression of the ethanolamine kinase activity did not affect the incorporation of [32P]orthophosphate into PtdEtn, indicating that the cellular levels of PtdEtn remained the same although the rate of synthesis increased. This observation was consistent with the enhanced glycerophosphoethanolamine levels we observed with the overexpression of EKI (Fig. 6).

EKI1 exhibited some minimal choline kinase activity (Fig. 2B). We transfected COS-7 cells with either EKI1 expression vector or a control vector, and after 48 h the transfected cells were labeled with [3H]choline to examine whether EKI1 activity modulated PtdCho biosynthesis. EKI1 overexpression caused a 2-fold increase in the incorporation of the label into phosphocholine but did not have an effect on the incorporation of the label into PtdCho (Fig. 7). These data suggested that the EKI1 enzyme did not modify PtdCho biosynthesis.

An alternative route for PtdEtn formation is the deacetylation of PtdSer. We transfected cells with EKI1 and labeled with [3H]serine in the presence of 32 μM ethanolamine in the culture medium to examine whether EKI1 acceleration of the CDP-ethanolamine pathway affected the rate of deacetylation of PtdSer. The amount of radiolabeled serine incorporated into PtdEtn was the same in control cells and in cells overexpressing EKI1 (20,805 ± 389 cpm/mg of cellular protein versus 23,773 ± 739 cpm/mg in cells transfected with pPJ96 and 20,789 ± 1217 cpm/mg in cells transfected with pAL10, respectively), indicating that increased rates of PtdEtn biosynthesis through the CDP-ethanolamine pathway did not affect the rate of PtdEtn biosynthesis via the deacetylation of PtdSer. The amount of the label incorporated into PtdSer was 34,202 ± 2390 cpm/mg protein in control cells, 33,600 ± 489 cpm/mg protein in cells transfected with pPJ96, and 28,966 ± 366 cpm/mg protein in cells overexpressing pAL10, suggesting that the overexpression of EKI1 also did not cause an accumulation of PtdSer.

**DISCUSSION**

The biochemical characterization of EKI1 reveals the existence of a specific EKI enzyme in mammalian cells. The human EKI cDNA encodes a protein with high specificity for ethanolamine (Figs. 4, A and B). The EKI1 preference for ethanolamine holds true in vivo as well where there is a dramatic stimulation of phosphoethanolamine formation (Fig. 6) and an increased PtdEtn radiolabeling (Fig. 5) in contrast to the lack of any significant change in PtdCho biosynthesis (Fig. 7). EKI1 may be identical to the mammalian ethanolamine-specific kinase activities described previously (19, 21). Weinhold and Rethy (21) partially purified an EKI activity with a molecular size of 36 kDa from rat liver that exhibited no CKI activity. Draus et al. (19) also reported the partial purification of a 42-kDa enzyme from human liver that had a relatively high ratio (~5) of EKI to CKI activities. The EKI1 clone reported in this paper encodes a protein with a
predicted molecular size of 49.7 kDa (Fig. 1) and may correspond to the enzymes described above.

We also report the existence of a second gene that is highly similar to EKI1, which we call EKI2. EKI1 and EKI2α proteins are 47% identical and 59% similar. EKI1 is 32% identical and 11% similar, whereas EKI2α is 29% identical and 38% similar to the Drosophila Eas protein. Based on the above comparison, it is difficult to definitely state which form is the mammalian homologue of the eas gene. However, there is emerging support for a family of EKI genes that is distinct from the CKI gene family, which encodes dual specificity enzymes. The existence of a mammalian ethanolamine-specific kinase has been a point of discussion due to the characterization of CKI activities that is similar to the one that controls cellular PtdCho levels (31). Increased glycerophosphoethanolamine was associated with stimulated PtdCho in response to EKI overexpression (Fig. 4). Earlier work from our laboratory indicated that excessive phospholipid synthesis is balanced by degradation to the glycerophosphate to maintain homeostatic levels of membrane lipid (31, 32). These results are consistent with the lack of an effect on the radiolabeling of PtdSer to PtdCho biosynthesis is not down-regulated by the activity of the CDP-ethanolamine pathway.

**Acknowledgments**—We thank Pam Jackson for excellent technical assistance and Charles Rock for critical comments on the manuscript.

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J. Biol. Chem. 2001, 276:2174-2179. doi: 10.1074/jbc.M008794200 originally published online October 23, 2000

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