

# Identification and characterization of human ethanolaminephosphotransferase1

Yasuhiro Horibata and Yoshio Hirabayashi<sup>1</sup>

Neuronal Circuit Mechanisms Research Group, Brain Science Institute, RIKEN, Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan

**Abstract** CDP-ethanolamine:diacylglycerol ethanolamine-phosphotransferase (EPT) catalyzes the transfer of phosphoethanolamine from CDP-ethanolamine to diacylglycerol to produce phosphatidylethanolamine (PE). To date, the dual specificity of choline/ethanolaminephosphotransferase (CEPT) has been recognized as the total activity responsible for the synthesis of PE via the CDP-ethanolamine pathway in human. We report here the identification and characterization of another human cDNA that encodes CDP-ethanolamine-specific human EPT (hEPT1). Through homology search, we found that human selenoprotein I contained the CDP-alcohol phosphatidyltransferase signature, a common motif conserved in phospholipid synthases. Bacterial expression of the cDNA in *Escherichia coli* demonstrated that the product specifically used CDP-ethanolamine as the phosphobase donor to produce PE with the activation by both Mn<sup>2+</sup> and Mg<sup>2+</sup>. RT-PCR and Northern blot analysis revealed that hEPT1 was ubiquitously expressed in multiple tissues, but in brain it was highly expressed in cerebellum. Here, we propose that in addition to previously identified CEPT, hEPT1 is involved in the biosynthesis of PE via the Kennedy pathway.—Horibata, Y., and Y. Hirabayashi. Identification and characterization of human ethanolaminephosphotransferase1. *J. Lipid Res.* 2007. 48: 503–508.

**Supplementary key words** phosphatidylethanolamine • selenoprotein I • phospholipid

Phosphatidylethanolamine (PE) in an abundant phospholipid in mammals, generally constituting 25% of cellular phospholipids (1). In various eukaryotic plasma membranes, aminophospholipids such as PE and phosphatidylserine (PS) reside in the inner leaflet, whereas choline-containing phospholipids such as phosphatidylcholine (PC) and sphingomyelin are localized mainly in the outer leaflet (1). Important roles for PE are as precursors of the glycosylphosphatidylinositol anchors, which are covalently attached to many cell surface proteins, and of *N*-acylethanolamine, which works as a neurotransmitter in the brain. Recent

studies also revealed that PE is involved in membrane fusion events (2), the regulation of lipid metabolism in *Drosophila* (3), and protein folding (4).

In mammals, the biosynthesis of PE occurs by two major routes. One is the mitochondrial decarboxylation of PS (5). The other is the CDP-ethanolamine pathway, as originally described by Kennedy and Weiss (6) in 1956. In this process, the phosphoethanolamine from CDP-ethanolamine is transferred to diacylglycerol with the release of CMP and the production of PE by ethanolamine-phosphotransferase (EPT). This enzyme is an integral membrane protein found primarily on endoplasmic reticulum membranes (7). Mutants of *Saccharomyces cerevisiae* defective in EPT activity were generated that permitted the identification of the EPT1 gene, which encodes for EPT. The EPT1 gene product, however, has dual specificity capable of using both CDP-ethanolamine and CDP-choline to produce PE and PC, respectively; that is, the enzyme possess cholinephosphotransferase (CPT) activity half as active as EPT activity (8). Based on sequence homology to the yeast sequence, a human cDNA that codes for a choline/ethanolaminephosphotransferase (CEPT) was identified (9). This enzyme (hCEPT1) also catalyzes both PC and PE production, possessing CPT activity >2-fold higher than EPT activity. After the discovery of hCEPT1, it was thought that in mammalian cells, the dual specificity of CEPT was totally responsible for the synthesis of PE via the CDP-ethanolamine pathway.

In this study, we found that a human expressed sequence tag (EST) clone annotated as selenoprotein I possesses the CDP-alcohol phosphatidyltransferase motif, a common motif conserved in phospholipid synthases. Expression of selenoprotein I in *Escherichia coli* showed CDP-ethanolamine-specific phosphatidyltransferase activity, in-

Abbreviations: CEPT, choline/ethanolaminephosphotransferase; CPT, cholinephosphotransferase; EPT, ethanolaminephosphotransferase; EST, expressed sequence tag; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

<sup>1</sup>To whom correspondence should be addressed.

e-mail: hirabaya@riken.jp

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dicating that the cDNA encodes a human EPT, now termed hEPT1. Here, we propose that in addition to previously identified hCEPT1, hEPT1 is involved in the biosynthesis of PE via the Kennedy pathway.

## MATERIALS AND METHODS

### Materials

Precoated Silica Gel 60 TLC plates were purchased from Merck. [ $\alpha$ - $^{32}$ P]dCTP was from MP Biomedicals. [*ethanolamine*-1,2- $^{14}$ C]CDP-ethanolamine (50 mCi/mmol) and [*methyl*- $^{14}$ C]CDP-choline (55 mCi/mmol) were from American Radiolabeled Chemicals. Diacylglycerol was from Doosan Biotech Co. All other reagents were of the highest purity available.

### Identification and cloning of hEPT1

A Basic Local Alignment Search Tool search was performed against the EST database with reference to the CDP-alcohol phosphatidyltransferase motif DG(X)<sub>2</sub>AR(X)<sub>8</sub>G(X)<sub>3</sub>D(X)<sub>3</sub>D. An EST clone (KIAA1724) (10) containing selenoprotein I cDNA was obtained from the Kazusa DNA Research Institute and sequenced. The full-length cDNA was amplified by PCR using Pyrobest DNA polymerase (Takara Bio) and subcloned into *EcoRI*/*XhoI* sites of the *E. coli* expression vector, pET23a (Novagen).

### RT-PCR and Northern blot analysis

For RT-PCR, first-strand cDNA from multiple human tissues (Human MTC Panel I; Clontech) was amplified by PCR using the upper (TGAGGCTGGTATGAACCTTTCCTGT) and lower (AGAGGCTACTCCTAGGTTTACCACT) primers to obtain a 406 bp fragment, according to the instructions of the manufacturer.

Premade Northern blots containing poly(A) RNA from eight different human brain tissues (Human Brain MTN Blot II; Clontech) were hybridized with random-primed  $^{32}$ P-labeled probes (the entire 1.2 kb coding region of hEPT1) at 68°C for 1 h and washed according to the manufacturer's instructions. Membranes were analyzed with the BAS-3000 imaging analyzer (Fujifilm, Tokyo, Japan).

### Expression and preparation of hEPT1 in *E. coli*

*E. coli* strain BL21(DE3)pLysS cells (Novagen) transformed with the expression vector was grown at 25°C in Luria-Bertani medium supplemented with 100  $\mu$ g/ml ampicillin and 35  $\mu$ g/ml chloramphenicol with shaking. Protein expression was induced by adding isopropyl  $\beta$ -D-thiogalactoside at a final concentration of 0.1 mM. After cultivation, cells were harvested and suspended in extraction solution (50 mM Tris-HCl buffer, pH 8.0, containing 5  $\mu$ g/ml leupeptin and 1 mM PMSF). The cell suspension was frozen at -20°C and then sonicated. After removal of cell debris by centrifugation (15,000 *g* for 10 min), the supernatant was ultracentrifuged at 100,000 *g* for 60 min. The membrane precipitated was suspended in the extraction solution and used as an enzyme source.

### Enzyme assays

Enzyme activity was determined using the membrane fraction of *E. coli* transformed with hEPT1. The reaction mixture contained 50 mM Tris-HCl buffer, pH 8.0, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 0.5 mM diacylglycerol, 0.002% (w/v) Tween 20, 5  $\mu$ g of membrane fraction, and 20  $\mu$ M CDP-ethanolamine (50 mCi/

mmol) or CDP-choline (55 mCi/mmol). After incubation at 37°C for 10 min, the reaction was stopped by adding 600  $\mu$ l of chloroform-methanol (1:1, v/v) followed by 300  $\mu$ l of 0.9% KCl. After centrifugation at 8,000 *g* for 5 min, the organic phase was dried and applied to TLC plates, which were then developed with chloroform-methanol-water (65:35:8, v/v/v). Radioactive phospholipids were analyzed with an imaging analyzer (BAS-3000) and quantified with Image Gauge version 3.0.

## RESULTS

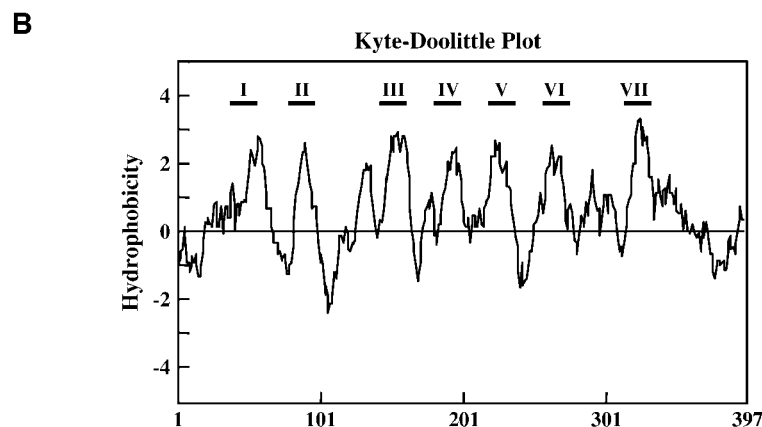
### Identification of hEPT1

The CDP-alcohol phosphatidyltransferase motif, DG(X)<sub>2</sub>AR(X)<sub>8</sub>G(X)<sub>3</sub>D(X)<sub>3</sub>D, is an amino acid sequence conserved in enzymes catalyzing the displacement of CMP from a CDP-alcohol by a second alcohol with formation of a phosphodiester bond and concomitant breaking of a phosphoride anhydride bond. It has been revealed that enzymes catalyzing the biosynthesis of phospholipids, such as CPT, CEPT, phosphatidylinositol synthase, cardiolipin synthase, and phosphatidylglycerol phosphate synthase, contain the CDP-alcohol phosphatidyltransferase motif. Through homology searches of human EST databases with reference to the motif, we found that a human cDNA annotated as selenoprotein I (GenBank accession number NM\_033505) completely conserved it, suggesting that this protein would function as CDP-alcohol phosphatidyltransferase (**Fig. 1A**). The homolog genes were also found in the EST database of *Mus musculus* (NP\_081928), *Xenopus tropicalis* (NP\_001004832), *Drosophila melanogaster* (NP\_788074), and *Caenorhabditis elegans* (NP\_491454), showing 88, 78, 43, and 39% identities to human cDNA at the amino acid level. Therefore, we considered the human selenoprotein I as a putative hEPT1. Kryukov et al. (11) reported that the first stop codon of the cDNA (TGA at positions 1,159–1,161) would function as a selenocysteine insertion codon using the computational program SECISearch (server at <http://genome.unl.edu/SECISearch.html>), annotated as selenoprotein I. Therefore, the open reading frame of the gene was composed of 1,194 nucleotides encoding 397 amino acids. From the deduced amino acid sequence, the molecular mass and isoelectric point of the hEPT1 were calculated to be 45,179 kDa and 6.13, respectively. A search for the transmembrane domain using a TMPred algorithm ([http://www.ch.embnet.org/software/TMPRED\\_form.html](http://www.ch.embnet.org/software/TMPRED_form.html)) (12) suggested that the predicted amino acid sequence contains seven transmembrane helices (amino acid positions 53–71, 85–103, 150–167, 190–209, 221–240, 261–278, and 323–342) (**Fig. 1B**). The hEPT1 sequence was mapped to a region of human chromosome 2.

**Figure 2** shows the alignment of predicted amino acid sequences of hEPT1, yeast EPT1 (yEPT1; NP\_011991), and hCEPT1 (NP\_006081). The open reading frame of hEPT1 showed 28% and 27% identity to that of yEPT1 and hCEPT1, respectively, at the amino acid level. The CDP-alcohol phosphatidyltransferase motif was all conserved in these enzymes.

**A**

1	ATGGCTGGCTACGAATACGTGAGCCCGGAGCAGCTGGCTGGCTTTGATAAGTACAAGTACAGTGCTGTGGATACCAATCCACTTTCTCTG	90
1	M A G Y E Y V S P E Q L A G F D K Y K Y S A V D T N P L S L	30
91	TATGTCATGCATCCATTCTGGAACACTATAGTAAAGTATTTCCTACTTGGCTGGCGCCCAATCTGATAACTTTTCTGGCTTTCTGCTG	180
31	Y V M H P F W N T I V K V F P T W L A P N L I T F S G F L L	60
181	GTCSTATTCAATTTCTGCTAATGGCATACTTTGATCCTGACTTTTATGCTCAGCACCAGTCCACAAGCAGTGCCTGACTGGSTTTGG	270
61	V V F N F L L M A Y F D P D F Y A S A P G H K H V P D W V W	90
271	ATTGTAGTGGGCATCCTCAACTTCGTAGCCTACACTCTAGATGGTGTGGACGGAAGCAAGCTCGCAGAACCAATTCTAGCATTCCCTTA	360
91	I V V G I L N F V A Y T L D G V D G K Q A R R T N S S T P L	120
361	GGGGAGCTTTTGTATCATGGCTGGATAGTGGTCATGTGTTACTTGTGTGACTGTTTATTCCATCTTTGGAAGAGGATCAACTGGT	450
121	G E L F D H G L D S W S C V Y F V V T V Y S I F G R G S T G	150
451	GTCAGTGTTTTGTCTTTATCTCCTGCTATGGGTAGTTTTGTTTTCTTTCATCCTGCTCCCACTGGGAAAAGTATAACACAGGGATTCTT	540
151	V S V F V L Y L L L W V V L F S F I L S H W E K Y N T G I L	180
541	TTCTGCCATGGGATAGACATTAGCCAGGTGACTATTTCTTTGTCTACATAGTGACTGCAGTTGTGGGAGTGGAGCCCTGGTATGAA	630
181	F L P W G Y D I S Q V T I S F V Y I V T A V V G V E A W Y E	210
631	CCTTCTGTGTTAATTTCTTATATAGAGACCTATTCACCTGCAATGATTATGGTGTGCAATTATGTGACTCTTCCAATGAGTTTATTA	720
211	P F L F N F L Y R D L F T A M I I G C A L C V T L P M S L L	240
721	AACTTTTTCAGAAGTATAAAAAAACACCTTGAAGCACTCAATTCAGTCTATGAAGCTATGGTTCCCTTATTTCTCCATGCTGTGCTGTC	810
241	N F F R S Y K N N T L K L N S V Y E A M V P L F S P C L L F	270
811	ATTTGTCTACAGCGTGGATCCTTTGGTCACCTTCAGATATTTAGAGCTACATCCTAGAGTATTCTACTTTATGGTGGAAACAGCCCTT	900
271	I L S T A W I L W S P S D I L E L H P R V F Y F M V G T A F	300
901	GCCAACAGTACATGTCAGCTGATTTGTCACCAATGAGTAGTACCCTGTCACACTTTGAATTGGTGTGGTTCCTCTCTCTCTGCTGTT	990
301	A N S T C Q L I V C Q M S S T R C P T L N W L L V P L F L V	330
991	GTCCTAGTGGTAAACCTAGGAGTAGCCCTCTTACGTTGAGAGCACTTCTCCTGATACATTAACAACCTGCTTTTACTCTGCCCCACATCCAT	1080
331	V L V V N L G V A S Y V E S I L L Y T L T T A F T L A H I H	360
1081	TATGGAGTACAGTGGTAAAGCAGCTGAGCAGCCATTTTCAGATTTACCCCTTCTCATTGAGGAAACCAAACTCAGAT <u>UGA</u> CTAGGAATG	1170
361	Y G V R V V K Q L S S H F Q I Y P F S L R K P N S D U L G M	390
1171	GAAGAAAAGAATATTGCCCTGTAA	1194
391	E E K N I G L *	398



**Fig. 1.** Nucleotide and deduced amino acid sequences (A) and hydropathy plot (B) of the human ethanolaminephosphotransferase (hEPT1). A: The deduced amino acid sequence is shown in one-letter code below the nucleotide sequence. Amino acid residues are numbered beginning with the first methionine, and the translation termination codon is denoted with an asterisk. The putative selenocysteine (U) insertion codon is boxed. B: The deduced amino acid sequence of the hEPT1 was analyzed by the method of Kyte and Doolittle for hydropathy plotting. The seven candidate transmembrane domains are indicated as I to VII.

### Expression and characterization of hEPT1

To clarify whether the putative hEPT1 has the ability to catalyze PE synthesis via the CDP-ethanolamine pathway, we expressed the cDNA in a bacterial expression system because prokaryotes including *E. coli* are devoid of the Kennedy pathway. As a result, ethanolamine phosphotransferase activity was observed in the membrane fraction of hEPT1-transformed cells, whereas no such activity was found in a vector control, indicating that selenoprotein I surely encodes EPT (**Fig. 3A**). It was interesting that in

contrast with yEPT1 and hCEPT1, hEPT1 showed no enzymatic activity catalyzing the production of PC by the use of CDP-choline. This result demonstrates that hEPT1 codes for a CDP-ethanolamine-specific phosphatidyltransferase.

Next, we analyzed the effect of cations on the activity of hEPT1 (**Fig. 3B**). This enzyme required millimolar levels of each cation for activity. The maximum activation was observed at the concentration of 5 mM  $Mn^{2+}$ , but it was inhibited at higher concentrations. In contrast, the optimum concentration of  $Mg^{2+}$  was 30 mM, and no sig-

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hEPT1 -----MAGYEYVSPQLAGFDKYYKSAVD
mEPT1 -----MAGYEYVSPQLSGFDKYYKSAVD
yEPT1 -----MGYFVPDISHIENLKSYYKQSED
hCEPT1 MSGHRSTRKRCGDSPVGFVGHMSTTGCVLNKLFLPTPLSRHQLKREEHRYQSAG
      . . . . .
      . . . . .

hEPT1 TNPLSLYVMHPFNTIIVKVFPTWLAFLNLTIFSGFLLVFNFLLMAYFDPDFYASAPGHKH
mEPT1 TNPLSLYIMHPFNTIIVKVFPTWLAFLNLTIFSGFLLVFNFLLLTYFDPDFYASAPGHKH
yEPT1 RSLVSKYFLKPFWQRFCHIFPTWMAFNIITLSGFAFIVINVLTVFYVDPLNLTDP----
hCEPT1 RSLLEPLMQG-YWEWLVRRVPSWIAPNLITIIIGLSINICTTLLLVFYCPTATEQAP----
      . . . . .
      . . . . .

hEPT1 VPDVWVIVVGIILNFVAYTLDGVDGKQARNTNSSTPLCELFHGLDWSWCVYFVVTVYSIF
mEPT1 VPDVWVIVVGIILNFVAYTLDGVDGKQARNTNSSTPLCELFHGLDWSWCVYFVVTVYSIF
yEPT1 --RWTYFSYALGVFLYQTFVGSQGVHARRINQSGPLCELFHSDAINSTLSIFIFASET
hCEPT1 --LWAYIACACGLFIYQSLDAIDGKQARNTNSSTPLCELFHGLDWSWCVYFVVTVYSIF
      * . . . . .
      . . . . .

hEPT1 GRGTGVSFVFLYLLLVVLFVFSFILSHWEKYNTGILFLPWGYDISQ--VTISFVYIVTAV
mEPT1 GRGTGVSFVFLYLLLVVLFVFSFILSHWEKYNTGVLFLPWGYDISQ--VTISFVYIVTAV
yEPT1 GMGFS---YNLMLSQFAMLTNFYLSWEEYHTHTLTLSEFSGPVEGILIVCVSLILTGI
hCEPT1 QLGTN---PDWFFCCFAGTFMFYCAHQWTVVSGTLRFGLIDVTEVQIFIIIMHLLAVIG
      * . . . . .
      . . . . .

hEPT1 VGVEAWYEPFLFNFLYRDFTAMIIGCALCVTLPMSELLNFRSYKNNILKLN-----
mEPT1 VGVEAWYEPFLFNFLYRDFTAMIIGCALCVTLPMSELLNFRSYKSNLTKHKS-----
yEPT1 YGKQVWHTYLFITIVGDKVIDVDTLDIVFSLAVGLVMNALSAKRNVDKYYRNSSTSSAN
hCEPT1 GPPFQWSMIPVLNIQMKIFPALCTVAGTIFSCNTYFRVIFTGGVKGNGSTIAG-----
      . . . . .
      . . . . .

hEPT1 -----VVEAMVPLFSPCLLFIILSTAWILWSPSDILELHPRVFYFMVGTAFANSTCQLIVC
mEPT1 -----VVEAMVPPFSPCLLFTLCTVWILWSPSDILEIHPRIFYFMVGTAFANITCQLIVC
yEPT1 NITQIQDSAIKGLLPPFAYYASIALLVWVQPSFITLS--PILSVGTGAFPTVGRIVC
hCEPT1 -----TSVLSPLFHIGSVITLAAMIYKSAVQLFEKHPCLYILTFGFVSAKITNKLVA
      . . . . .
      . . . . .

hEPT1 QMSSTRCPNLNWLVP----LFLVVLVNLGVAS-YVESILLYTLTAFTLAHIHYGVR
mEPT1 QMSSTRCPNLNWLVP----LLLVVAIVVGAATSRLSEALLYTLTAFTLAHIHYGVQ
yEPT1 HLTKQSPFMFNAPMLIPLCQIVLYKICLSLWGIENKIVFALSWSLGFGLSLGVHIMFMND
hCEPT1 HMTKSEMHLHDFAFIG-----PALLFLDQYFNSFIDEYIVLWIALVFSFFDLIRYCVS
      . . . . .
      . . . . .

hEPT1 VVKQLSSHFIYPPSLRKPNSDULGMEEKNIGL
mEPT1 VVKQLSRHPQIYPPSLRKPNSDULGMEEKNIGL
yEPT1 IIEHFEYLDVYALSIRKSKLT-----
hCEPT1 VCNQIASHLHIVFRIVKSTAHSNHH-----
      . . . . .
      . . . . .
    
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Fig. 2. Alignment of hEPT1 with yeast EPT1 and human choline/ethanolaminophosphotransferase (CEPT1). The predicted amino acid sequences of hEPT1, mouse EPT1 (mEPT1; NP\_081928), *Saccharomyces cerevisiae* EPT1 (yEPT1; NP\_011991), and human CEPT1 (hCEPT1; NP\_006081) were aligned using the ClustalW algorithm. Identical amino acids are indicated by asterisks, and conserved and partially conserved amino acids are indicated by double dots and single dots, respectively. Gaps inserted into the sequences are indicated by dashed lines. Amino acid residues conserved in the CDP-alcohol phosphatidyltransferase motif are shown on a black background.

nificant inhibition was observed at higher concentrations up to 120 mM (data not shown).

Next, we investigated the kinetics of hEPT1 with CDP-ethanolamine and CDP-choline as the substrates in the

presence of 5 mM Mn<sup>2+</sup>. Figure 3C shows the substrate saturation curve. When the concentration of CDP-choline in the reaction mixture was >25 μM, subtle PC was produced, yielding <3% PE. This result indicated that

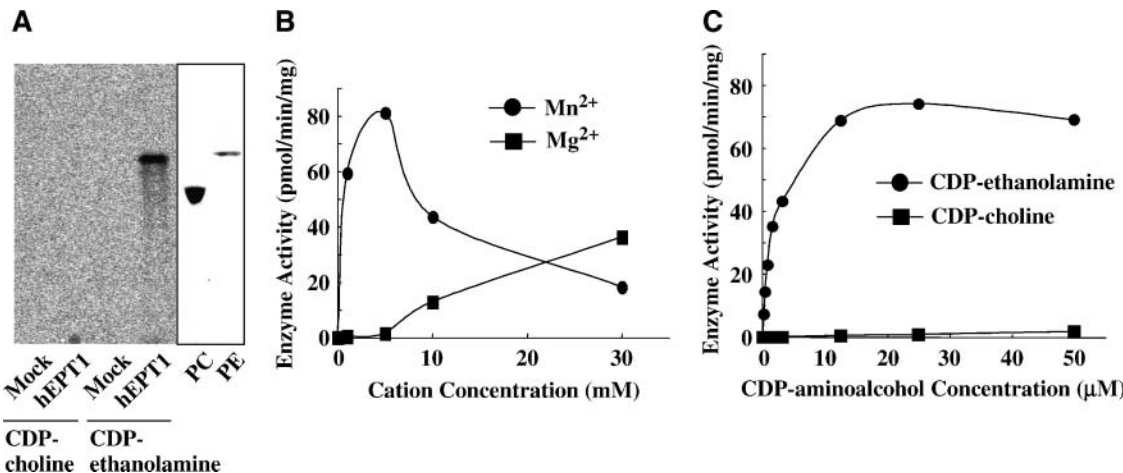


Fig. 3. Expression and characterization of hEPT1. A: Enzyme activities in the membrane fractions of mock-transfected (pET23a) and hEPT1-transfected *E. coli* were measured using CDP-ethanolamine or CDP-choline as the substrate in the presence of 5 mM Mn<sup>2+</sup>, as described in Materials and Methods. PC, phosphatidylcholine; PE, phosphatidylethanolamine. B: EPT activities in the membrane fraction of hEPT1-expressing cells were determined in the presence of various concentrations of Mn<sup>2+</sup> or Mg<sup>2+</sup>. Data shown are means from a representative experiment. C: EPT and cholinephosphotransferase activities in the membrane fraction of hEPT1-expressing cells were measured using various concentrations of CDP-ethanolamine and CDP-choline in the presence of 5 mM Mn<sup>2+</sup>. Data shown are means from a representative experiment.

hEPT1 could, but very weakly, use CDP-choline as a substrate. Apparent  $K_m$  and  $V_{max}$  values of this enzyme for CDP-ethanolamine were estimated to be 1.8  $\mu\text{M}$  and 76.3 pmol/min/mg.

### Tissue expression pattern of hEPT1

To assess the tissue distribution of hEPT1, RT-PCR was conducted using a multiple human tissue cDNA. As shown in Fig. 4A, the gene seemed to be expressed in all tissues. The expression of hEPT1 was abundant in brain, placenta, liver, and pancreas, followed by heart, skeletal muscle, lung, and kidney. Northern blots of various parts of human brain revealed that the hEPT1 gene was strongly expressed in cerebellum, followed by the occipital pole and the frontal lobe. The transcript size of hEPT1 was estimated to be 7.9 kb. This is approximately consistent with the size of mRNA for selenoprotein I (8,091 bp) as published in the GenBank database (NM\_033505).

### DISCUSSION

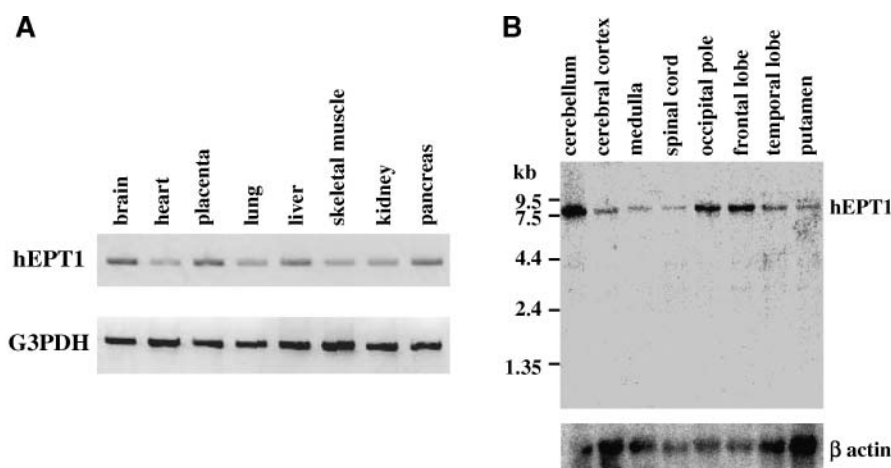
In mammalian cells, the biosynthesis of PE occurs by two different pathways. The first is the decarboxylation of PS in mitochondria by PS decarboxylase (5). The other is the CDP-ethanolamine pathway, as originally reported by Kennedy and Weiss (6). In this process, PE is synthesized by EPT, which catalyzes the transfer of ethanolamine from CDP-ethanolamine to diacylglycerol. To date, hCEPT1 has been thought to be the only enzyme involved in PE synthesis via the CDP-ethanolamine pathway in human. This enzyme, however, has dual specificity capable of using both CDP-ethanolamine and CDP-choline to produce PE and PC, respectively. In this study, we found that a cDNA annotated as selenoprotein I possesses EPT activity; this cDNA is termed hEPT1. Interestingly, in contrast to previously reported EPT enzymes such as hCEPT1

and  $\gamma$ EPT1, hEPT1 specifically used CDP-ethanolamine as the phosphobase donor. This is the first report of the gene identification of CDP-ethanolamine-specific EPT.


Another CDP-ethanolamine-specific EPT, showing a  $K_m$  value for CDP-ethanolamine  $\sim 70$  times smaller than that for CDP-choline, has also been purified to near homogeneity from bovine liver microsomes (13). A 38 kDa protein was demonstrated to show  $\text{Mn}^{2+}$ -dependent EPT activity, whereas such activity was not detected in the presence of  $\text{Mg}^{2+}$ . However, this is inconsistent with hEPT1, because this enzyme was activated by both cations. It may be possible that there is another CDP-ethanolamine-specific EPT in animals. Confirmation of the amino acid sequence of the purified protein will resolve this question. By contrast, the cation requirement of hEPT1 is similar to that of hCEPT1. The EPT activity of hCEPT1 was reported to be activated by both  $\text{Mn}^{2+}$  and  $\text{Mg}^{2+}$  and inhibited at higher  $\text{Mn}^{2+}$  concentrations.

Here, we found that in brain, hEPT1 is strongly expressed in cerebellum. Interestingly, 3-phosphoglycerate dehydrogenase, a key enzyme for L-serine biosynthesis, is also abundantly expressed in cerebellum (14). Because L-serine is an essential precursor for the generation of ethanolamine (i.e., the biosynthesis of the compound in animals is only derived from the breakdown of PS or sphingolipids) (15), there may be abundant CDP-ethanolamine in cerebellum and the biosynthesis of PE by hEPT1 may also be more highly active.

Recently, knockout mice disrupted in PS decarboxylase were generated (16). The mice showed lethality between days 8 and 10 of embryonic development and abnormal morphology in mitochondria. Interestingly, however, the PE content of wild-type and knockout mice was almost the same. It was also revealed that PE synthesis via the CDP-ethanolamine pathway was increased in the knockout mice. These results suggest that the total content of cellular PE is regulated by both PS decarboxylation and



**Fig. 4.** Expression pattern of hEPT1 in multiple human tissues. A: RT-PCR analysis of hEPT1. First-strand cDNA from various human tissues was amplified using hEPT1-specific primers. Glyceraldehyde 3-phosphate dehydrogenase (G3PDH) expression was also analyzed for an internal control. B: Poly(A) RNA from various brain parts was hybridized with radiolabeled probes prepared from the entire cDNA of hEPT1. The positions of molecular mass markers are indicated at left.

CDP-ethanolamine phosphatidyltransfer, with a cross-talk mechanism to maintain phospholipid homeostasis. Study of whether or not PE synthesis via the CDP-ethanolamine pathway is also essential in mice is awaited, and our study will facilitate further research into the biological functions of PE. 

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