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Biochimica et Biophysica Acta 1418 (1999) 239–244

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Rapid report

Identity of the *F52F12.1* gene product in *Caenorhabditis elegans* as an organic cation transporter

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Received 19 January 1999; accepted 27 January 1999

Abstract

We describe here the cloning and functional characterization of an organic cation transporter from *Caenorhabditis elegans* (CeOCT1). The CeOCT1 cDNA is 1826 bp long and codes for a protein of 568 amino acids. The *oct1* gene is ~3.2 kb in size and consists of 12 exons. The location of this gene corresponds to the *F52F12.1* gene locus on chromosome I. The predicted protein contains 12 putative transmembrane domains. It exhibits significant homology to mammalian OCTs. When expressed in mammalian cells, CeOCT1 induces the transport of the prototypical organic cation tetraethylammonium. The Michaelis–Menten constant for this substrate is 80 ± 16 μM. The substrate specificity of CeOCT1 is broad. This represents the first report on the cloning and functional characteristics of an organic cation transporter from *C. elegans*.

Keywords: Organic cation transporter; Primary structure; Gene structure; Drug transport; (*Caenorhabditis elegans*)

Polyspecific organic cation transporters mediate the excretion of a wide variety of positively charged endogenous metabolites and xenobiotics [1,2]. These transporters are expressed in the kidney, liver and intestine. This tissue distribution pattern is consistent with the function of the organic cation transporters in the elimination of cationic xenobiotics from the body. In recent years, several organic cation transporters (OCTs) have been cloned and functionally characterized from several animal species [3,4], providing an insight into the molecular nature and functional mechanism of these transporters. The first member of the fast growing organic cation transporter family to be cloned was OCT1 [5]. Subsequently, OCT2 and OCT3 were cloned [6,7]. These three OCTs form a subgroup within the OCT family and the predicted amino acid sequences exhibit ~70% similarity among them. Another subgroup of the OCT family consists of OCTN1 and OCTN2 [8,9], which are much closer to each other than to the members of the first subgroup at the level of primary structure. All of the organic cation transporters cloned thus far mediate the transport of the prototypical organic cation tetraethylammonium (TEA). Interestingly, OCTN2 has recently been shown to function also as a Na^+-dependent carnitine transporter [10,11].

Caenorhabditis elegans is being increasingly recog-

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0005-2736/99/$ – see front matter
PII: S0005-2736(99)00020-6

nized as a very useful model system to study the function of various genes expressed in higher animals. The complete structure of the *C. elegans* genome has been completed recently [12]. Database searches of the *C. elegans* genomic DNA sequence for genes homologous to mammalian organic cation transporters identified a putative candidate. The location of this gene corresponds to the *F52F12.1* gene locus on chromosome I. In this paper, we report on the cloning of the full-length cDNA coding for this *C. elegans* protein and on the functional characterization of the protein by heterologous expression of the cDNA in mammalian cells. The functional studies have established the cloned *C. elegans* cDNA as an organic cation transporter with a broad specificity for structurally diverse cationic compounds.

Total RNA was isolated from *C. elegans* with Trizol reagent (Life Technologies) according to manufacturer's protocol. Poly(A)$^+$ RNA was then prepared by affinity chromatography on an oligo-dT cellulose column (Life Technologies). The integrity of the RNA sample was checked by formaldehyde–agarose gel electrophoresis.

A pair of PCR primers specific for the putative *C. elegans* organic cation transporter encoded by the gene in the *F52F12.1* locus was designed: 5′-GTTTCTGCAATGTTTATGCC-3′ (forward primer) and 5′-TCGACTACTTCTCCGTCTTC-3′ (reverse primer). Reverse transcription–polymerase chain reaction (RT–PCR) was conducted using *C. elegans* poly(A)$^+$ RNA with Geneamp RNA-PCR kit (Perkin Elmer). A single product was obtained with an estimated size of 450 bp as predicted by the primers. The PCR product was genecleaned and cloned into pGEM-T vector (Promega). The cDNA insert was sequenced by the dideoxynucleotide chain termination method for confirmation of its identity.

The SuperScript Plasmid system (Gibco BRL) was used to establish the directional cDNA library using the poly(A)$^+$ RNA isolated from *C. elegans* [13]. The cDNA probe obtained by RT–PCR was labeled with [α-^{32}P]dCTP using the Ready-to-go oligolabeling kit (Pharmacia). The *C. elegans* cDNA library was screened with the probe under medium stringency conditions. Hybridization was carried out at 65°C for 20 h in a solution containing 5×SSPE (1×SSPE = 0.15 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA), 5×Denhardt's solution, 0.5% SDS, and 100 μg/ml of denatured salmon sperm DNA. Post-hybridization washing was done as described earlier [7,9], which involved extensive washes with 3×SSPE/0.5% SDS at room temperature. Positive clones were identified and the colonies purified by secondary screening.

Functional analysis of the cloned cDNA was carried out by heterologous expression in human retinal pigment epithelial (HRPE) cells using the vaccinia virus expression technique [14]. This cell line has been shown to be much better suited than the more widely used HeLa cells for functional characterization of organic cation transporters [15]. The cloned cDNA is present in pSPORT vector under the control of T7 promoter and expression of the cDNA in mammalian cells is mediated by a recombinant vaccinia virus carrying the gene for T7 RNA polymerase. Transport of [^{14}C]TEA in HRPE cells expressing the CeOCT1 cDNA was measured as described previously [7,9]. The transport buffer was 25 mM Tris–Hepes (pH 8.5), supplemented with 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose. When the influence of pH on transport was studied, buffers of different pH were prepared by appropriately mixing two buffers: 25 mM Mes–Tris (pH 5.5) and 25 mM Tris–Hepes (pH 8.5). Both buffers contained all other salt supplements and glucose. Cells transfected with pSPORT vector alone was used to determine endogenous organic cation transport activity. The transport activity in cDNA-transfected cells was adjusted for the endogenous transport activity to calculate cDNA-specific activity.

Two positive clones were isolated by screening of $\sim 5\times10^5$ colonies of the *C. elegans* cDNA library, with cDNA inserts of 1.8 and 1.3 kbp in size. Sequencing of the clones indicated that the 1.8 kbp cDNA, designated CeOCT1, was a full-length clone whereas the 1.3 kbp cDNA was a truncated CeOCT1.

The nucleotide sequence (GenBank accession no. AF110415) and the deduced amino acid sequence of CeOCT1 are given in Fig. 1. The cDNA is 1826 bp long with a 1707 bp long open reading frame (including termination codon). The cDNA codes for a protein of 568 amino acids. Hydropathy analysis of the predicted amino acid sequence indicates that the protein possesses 12 putative transmembrane domains.

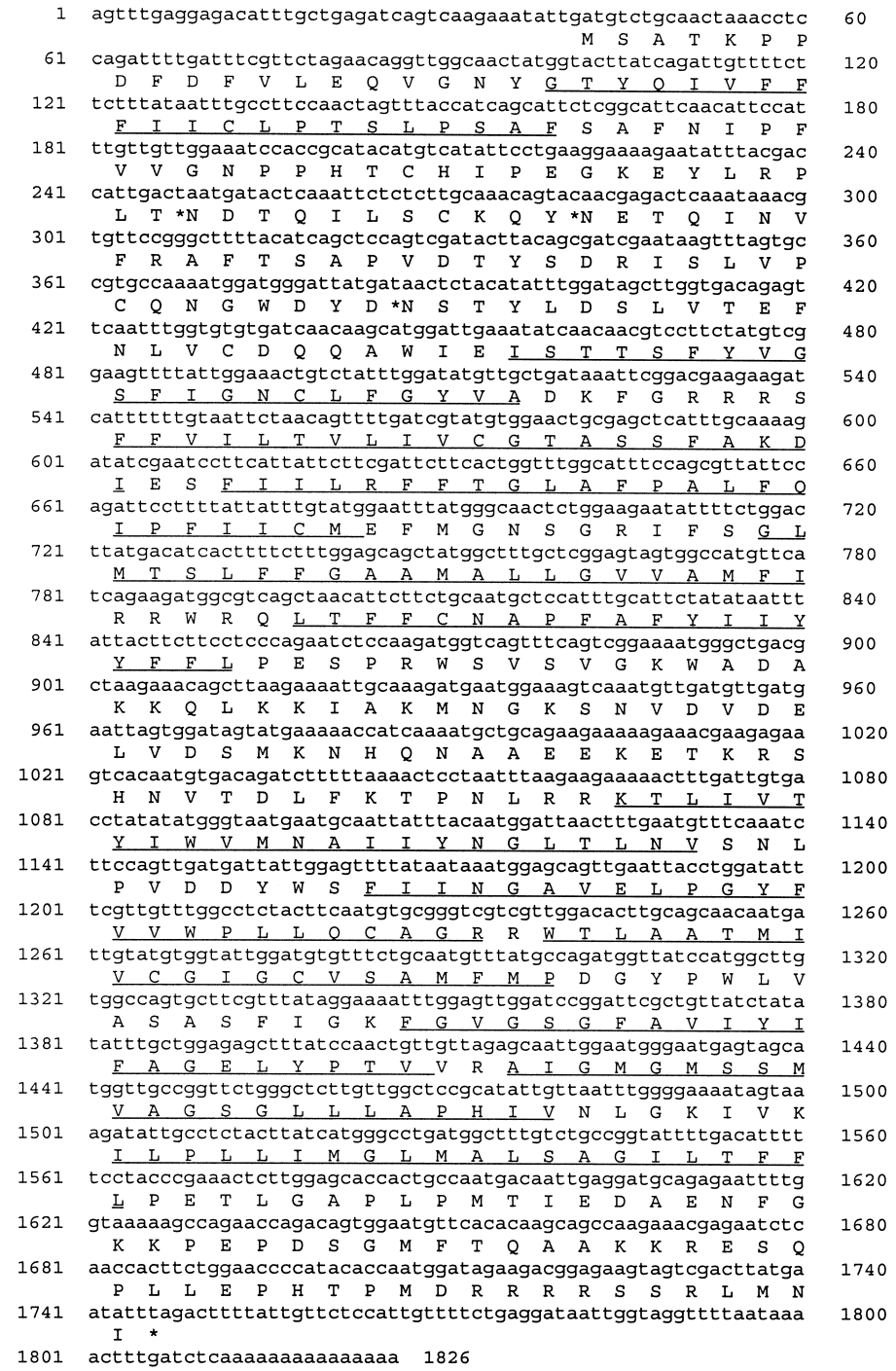

Fig. 1. Nucleotide sequence of CeOCT1 cDNA and predicted amino acid sequence. Putative transmembrane domains are underlined and putative *N*-glycosylation sites are indicated by asterisks.

When modeled similar to the other known mammalian organic cation transporters, both the amino terminus and the carboxy terminus of the CeOCT1 protein face the cytoplasmic side of the membrane. There is a long extracellular loop consisting of 98 amino acids between the first two transmembrane domains. This loop contains three potential sites for *N*-glycosylation (Asn-70, Asn-81, and Asn-116).

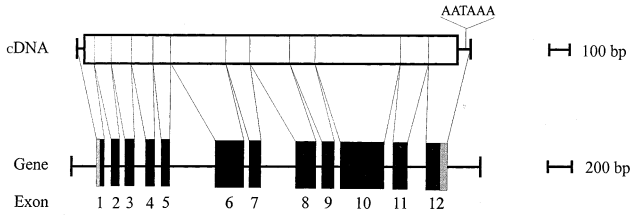

Fig. 2. Exon–intron organization of the gene encoding CeOCT1 and its organizational relationship to the CeOCT1 cDNA. Black boxes represent the protein coding regions of the exons, and stippled boxes represent the 5′ untranslated region in exon 1 and 3′ untranslated region in exon 12.

Table 1
Comparison of amino acid sequence between CeOCT1 and mammalian OCTs

Mammalian OCT	CeOCT1	
	% Identity	% Similarity
Rat OCT1	29	47
Rat OCT2	29	46
Rat OCT3	27	45
Rat OCTN1	30	51
Rat OCTN2	29	49

The predicted molecular mass of the protein is 63.5 kDa.

At the level of amino acid sequence, CeOCT1 is closely related to the mammalian organic cation transporters (27–30% identity; 45–51% similarity) (Table 1). A comparison of the CeOCT1 cDNA sequence with the complete *C. elegans* genomic sequence has enabled us to deduce the exon–intron organization of the gene (Fig. 2). The gene consists of 12 exons and 11 introns. All exon–intron boundaries conform to consensus donor–acceptor sequences (gt/ag) for RNA slicing.

In order to establish the functional identity of CeOCT1 as an organic cation transporter, we employed the vaccinia virus expression system to functionally express the CeOCT1 cDNA in HRPE cells. Cells transfected with empty pSPORT vector served as the control. Transport measurements were made at room temperature using [^{14}C]TEA. Fig. 3 describes the time course of TEA transport in cDNA-transfected cells versus vector-transfected cells. The transport was several-fold higher in cells expressing the CeOCT1 cDNA compared with vector-transfected cells. The transport was linear even up to 2 h. A 60-min incubation was used for subsequent characterization of the transport function. With this incubation period, TEA transport in CeOCT1 cDNA-transfected cells was 8-fold higher compared with transport in cells transfected with vector alone. The cDNA-mediated transport of TEA was found to be highly pH-sensitive, the activity becoming increasingly higher as the pH of the transport buffer was changed from 5.5 to 8.5 (Fig. 4). Under similar conditions, the endogenous TEA transport activity

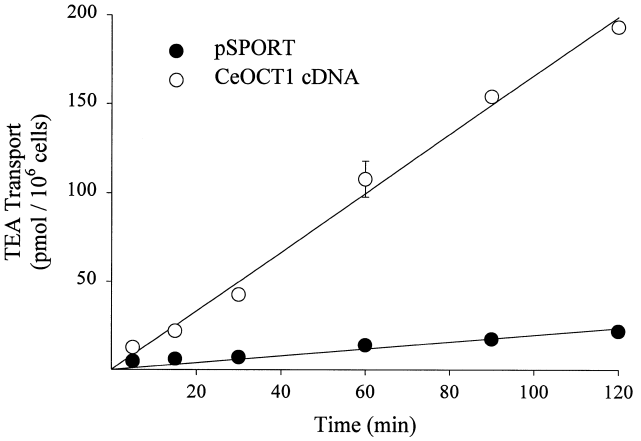

Fig. 3. Time course of TEA transport in HRPE cells transfected with either vector alone (●) or CeOCT1 cDNA (○). Transport was measured at pH 8.5. Concentration of [^{14}C]TEA was 15 μM.

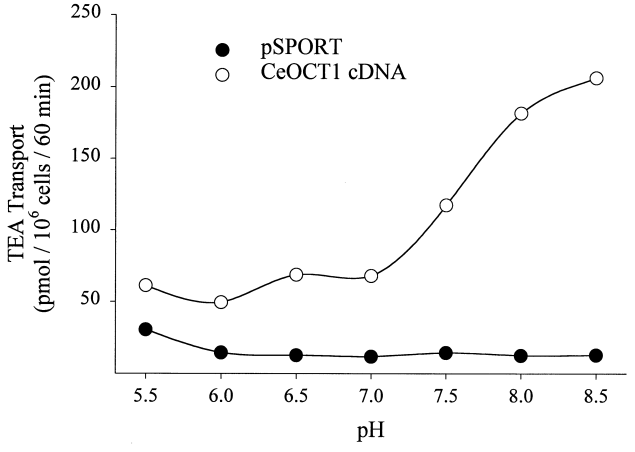

Fig. 4. Influence of pH on the transport function of CeOCT1. HRPE cells were transfected with either vector alone (●) or CeOCT1 cDNA (○). Transport of [^{14}C]TEA (25 μM) was measured at different pH (5.5–8.5) using a 60-min incubation time.

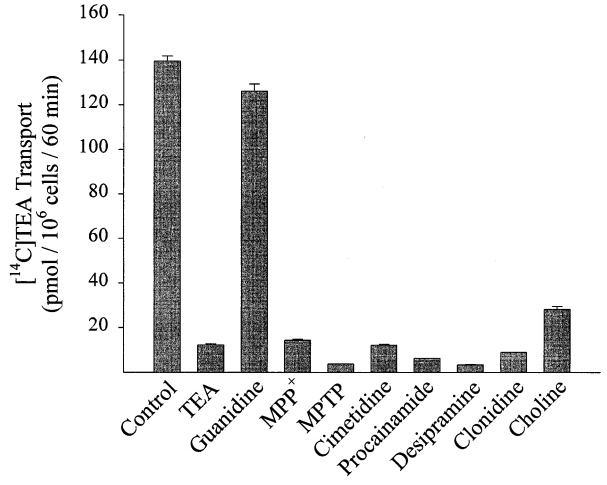

Fig. 5. Substrate specificity of CeOCT1. HRPE cells were transfected with either vector alone or CeOCT1 cDNA. Transport of [^{14}C]TEA (20 μM) was measured at pH 8.5 using a 60-min incubation time in the presence or absence of unlabeled inhibitors (2.5 mM). Transport measured in cells transfected with vector alone was subtracted from transport measured in cDNA-transfected cells to calculate CeOCT1-specific transport. TEA, tetraethylammonium; MPP^+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

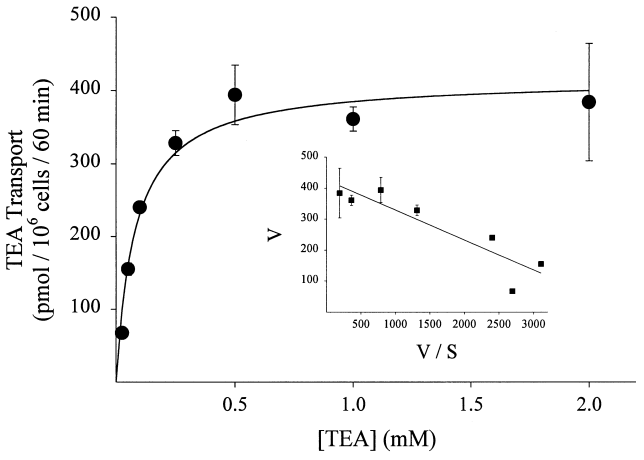

Fig. 6. Saturation kinetics of CeOCT1-mediated TEA transport. Transport was measured in CeOCT1-expressing HRPE cells over a TEA concentration range of 25 μM–2 mM. Incubation time was 60 min. Transport measured in cells transfected with vector alone was subtracted from transport in cDNA-transfected cells to calculate CeOCT1-mediated transport. Inset: Eadie–Hofstee plot. V, TEA transport in pmol/10^6 cells per 60 min; S, TEA concentration in mM.

measured in vector-transfected cells was not influenced by pH.

The substrate specificity of the cDNA-induced transport activity was investigated by assessing the effect of various organic cations on the transport of [^{14}C]TEA in cDNA-transfected cells (Fig. 5). The transport of [^{14}C]TEA (20 μM) was almost completely inhibited by all organic cations (2.5 mM) tested except guanidine, showing that CeOCT1 cDNA encodes an organic cation transporter with a broad substrate specificity.

Fig. 6 describes the saturation kinetics of TEA transport in HRPE cells mediated by CeOCT1. Transport was measured in vector-transfected cells and in cDNA-transfected cells with varying concentrations of TEA (25 μM–2 mM). The transport in control cells was subtracted from the transport in cDNA-transfected cells to determine the cDNA-specific transport which was then used in kinetics analysis. The CeOCT1-mediated TEA uptake was saturable, with a Michaelis–Menten constant of 80 ± 16 μM.

This represents the first report on the cloning and functional characterization of an organic cation transporter from *C. elegans*. The cloned CeOCT1 recognizes structurally diverse organic cations as substrates. Many of these substrates are pharmacologically active drugs or neurotoxins. Therefore, the CeOCT1 might play a role in the elimination of xenobiotics from the organism. The findings that MPTP and MPP^+ are substrates for the CeOCT1 are interesting because these compounds are known to be specific toxins for dopaminergic neurons due to the concentrative accumulation of these toxins inside the neurons via the dopamine transporter [16]. There is evidence for dopaminergic neurotransmission and expression of a dopamine transporter in *C. elegans* [17–19], suggesting that MPP^+ and MPTP may have neurotoxic effects in this organism as has been found in higher animals. The OCT1 might play a protective role in eliminating these and other toxins from the organism. A more recent database search has indicated that there may be additional genes homologous to OCT1 in *C. elegans*, raising the possibility that the OCT1 reported in this paper may not be the only organic cation transporter in this organism. It is very likely that *C. elegans* expresses multiple organic cation transporters with overlapping substrate specificity which work together to eliminate a broad spectrum of structurally diverse xenobiotics and environmental toxins. The organic cation transporters are

not the only transporters involved in the elimination of xenobiotics in *C. elegans*. This organism also expresses proteins similar to mammalian P-glycoprotein which is known to transport actively a wide variety of xenobiotics out of the cell [20,21].

C. elegans is a free-living soil nematode and is exposed to natural toxins in the soil produced by microorganisms and plants. The soil also contains toxic chemicals present in the environment. The nematode is likely to have transport mechanisms to eliminate these toxic compounds from the body. In animals, the kidney, intestine and liver possess the ability to excrete drugs and toxins. These organs express various drug transporting systems including the organic cation transporters and the P-glycoprotein. *C. elegans* has neither liver nor kidney. Instead, the nematode contains an excretory system consisting of four cells which probably functions in the elimination of environmental toxins. The nematode intestine, consisting of a tube of 20 cells, may also play a role in the elimination of toxins. The P-glycoproteins are present in the lumenal membrane of the cells in the excretory system and intestine in *C. elegans* [20,21]. It is likely that the cloned OCT1 also exhibits a similar distribution pattern consistent with its suggested role in the elimination of toxins.

Acknowledgements

This work was supported by National Institutes of Health Grant DA 10045. The authors thank Vickie Mitchell for excellent secretarial assistance.

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