

Identification of the Human Mitochondrial Oxodicarboxylate Carrier

BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION, TISSUE DISTRIBUTION, AND CHROMOSOMAL LOCATION*

Received for publication, October 20, 2000, and in revised form, November 15, 2000
Published, JBC Papers in Press, November 16, 2000, DOI 10.1074/jbc.M009607200

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In *Saccharomyces cerevisiae*, the genes ODC1 and ODC2 encode isoforms of the oxodicarboxylate carrier. They both transport C5-C7 oxodicarboxylates across the inner membranes of mitochondria and are members of the family of mitochondrial carrier proteins. Orthologs are encoded in the genomes of *Caenorhabditis elegans* and *Drosophila melanogaster*, and a human expressed sequence tag (EST) encodes part of a closely related protein. Information from the EST has been used to complete the human cDNA sequence. This sequence has been used to map the gene to chromosome 14q11.2 and to show that the gene is expressed in all tissues that were examined. The human protein was produced by overexpression in *Escherichia coli*, purified, and reconstituted into phospholipid vesicles. It has similar transport characteristics to the yeast oxodicarboxylate carrier proteins (ODCs). Both the human and yeast ODCs catalyzed the transport of the oxodicarboxylates 2-oxoadipate and 2-oxoglutarate by a counter-exchange mechanism. Adipate, glutarate, and to a lesser extent, pimelate, 2-oxopimelate, 2-aminoadipate, oxaloacetate, and citrate were also transported by the human ODC. The main differences between the human and yeast ODCs are that 2-aminoadipate is transported by the former but not by the latter, whereas malate is transported by the yeast ODCs but not by the human ortholog. In mammals, 2-oxoadipate is a common intermediate in the catabolism of lysine, tryptophan, and hydroxylysine. It is transported from the cytoplasm into mitochondria where it is converted into acetyl-CoA. Defects in human ODC are likely to be a cause of 2-oxoadipate acidemia, an inborn error of metabolism of lysine, tryptophan, and hydroxylysine.

In mammals, 2-oxoadipate is produced from lysine in the cytosol of cells via the saccharopine and the pipercolic acid pathways. Catabolites of hydroxylysine and tryptophan enter these pathways as 2-aminoadipic- δ -semialdehyde and 2-oxoadipate, respectively. In the matrix of mitochondria, 2-oxoadipate is decarboxylated to glutaryl-CoA by the 2-oxoadipate dehydrogenase complex and then converted to acetyl-CoA.

Over the years, several patients with 2-oxoadipate acidemia, an in-born error of metabolism of lysine, tryptophan, and hydroxylysine, have been reported, most of them slightly to deeply mentally retarded with hypotonia or seizures (1–7). It was speculated without experimental verification that their abnormal levels of serum and urinary 2-oxoadipate might arise from a defect in 2-oxoadipate dehydrogenase. As this enzyme is found in the matrix of mitochondria, and 2-oxoadipate is produced in the cytosol (8), impaired transport of 2-oxoadipate into the organelle provides an alternative explanation, but until the present work no such transport function had been demonstrated in man.

In this paper, the identification of the human 2-oxoadipate mitochondrial carrier (ODC)¹ is described. It is based on two isoforms, ODC1 and ODC2 encoded in the genome of *Saccharomyces cerevisiae*, that transport the oxodicarboxylates 2-oxoadipate and 2-oxoglutarate across the inner membranes of mitochondria (9). However, yeast and man are too distant phylogenetically for these isoforms to provide a basis for direct cloning of the mammalian counterpart. Therefore, orthologs were sought and detected in *Caenorhabditis elegans* and *Drosophila melanogaster* and used to bridge between yeast and man. In this way, a human EST was identified that encodes a fragment of a related protein. It provided information to complete the human cDNA sequence. The encoded protein is 299 amino acids long and has the characteristic features of the family of mitochondrial carrier proteins (10–13). It was overexpressed in *Escherichia coli*, reconstituted into phospholipid vesicles, and shown to have transport specificity and other biochemical properties similar to those of the recombinant yeast ODC isoforms, including transport of 2-oxoadipate.

* This work was supported by the Consiglio Nazionale delle Ricerche Target Project "Biotechnology," by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST), by the Piano Biomedicina, Progetto No. 1 Cluster C04 L.488/92, by MURST 5%-Consiglio Nazionale delle Ricerche program Biomolecole per la Salute Umsna L. 95/95, and by the European Social Fund. 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.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ278148 and AJ289714 (for the human and rat ODC cDNAs, respectively).

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¹ The abbreviations used are: ODC, oxodicarboxylate carrier protein; bp, base pair(s); EST, expressed sequence tag; FISH, fluorescence *in situ* hybridization; kb, kilobase(s); nt, nucleotide; PAGE, polyacrylamide gel electrophoresis; RT-PCR, reverse transcription polymerase chain reaction.

EXPERIMENTAL PROCEDURES

Sequence Search and Analysis—Data bases of the genomes of *C. elegans* and *D. melanogaster* at the Sanger Center (Hinxton, United Kingdom) and at the National Center for Biotechnology Information (Washington, D. C.) were screened with the sequences of the *S. cerevisiae* ODC proteins (9) using the program BLASTP. The NCBI nonredundant EST human data base was probed with the program TBLASTN. Amino acid sequences were aligned with ClustalW (version 1.7).

Human and Rat cDNAs for the ODC—Touchdown PCRs (14) were performed with adaptor-ligated double-stranded human liver cDNA (1 ng, CLONTECH), as described previously (15, 16). The full-length cDNA sequence was obtained in two PCR reactions of 5'- and 3'-extension using the adaptor primers AP1 and AP2 (CLONTECH) and a primer set 1F, 2F, 1R, and 2R (Fig. 1) designed from the nucleotide sequence of the human EST, R29313. The PCR products were identified, recovered from agarose gels, cloned into the pCR2.1 topo-vector (Invitrogen), and sequenced. The cDNA for rat ODC was amplified similarly with oligonucleotides based on the human cDNA sequence.

Cytogenetics—Human metaphase chromosome spreads were obtained from phytohemagglutinin-stimulated peripheral lymphocytes. A probe for FISH analysis was made by PCR amplification of human genomic DNA using primers corresponding to nt 916–939 (forward sense) and nt 1191–1212 (reverse sense) of the human cDNA for ODC (see Fig. 1). The resulting 4.2-kb fragment was cloned in the pCR2.1 topo-vector and sequenced to confirm its identity. The FISH experiments were performed as described previously (17).

Expression Analysis by RT-PCR—Total RNAs (2 μ g) were extracted and reverse-transcribed with the Gene Amp RNA PCR Core kit (PerkinElmer Life Sciences) using either random hexamers or oligo(dT)₁₆ as primers (final volume, 40 μ l). A 230-bp fragment of the ODC cDNA was then amplified from the reverse transcription reaction products (20 μ l) by 35 cycles of PCR using oligonucleotides RT1F (nt 662–682) and RT1R (nt 869–892) as forward and reverse primers, respectively (Fig. 1). The products were probed with the radiolabeled oligonucleotide RT1P (nt 804–825; Fig. 1). As a control, a 384-bp β -actin fragment was amplified from the remainder of the reverse transcription products (18).

Bacterial Overexpression of the Human ODC—The coding regions for the human and rat ODCs were amplified from human and rat liver cDNAs (1 ng) by 35 cycles of PCR. The forward and reverse primers in these reactions corresponded to nt 311–331 and 1188–1210 of the human ODC cDNA (see Fig. 1) and to nt 100–120 and 976–996 of the rat ODC cDNA (deposited as GenBank™ accession number AJ289714). The forward and reverse primers carried an *Nde*I and a *Xho*I site, respectively, at their 5'-ends. The 0.9-kb products were gel-purified and cloned into the pRUN expression vector. Transformants of *E. coli* DH5 α cells were selected on ampicillin (100 μ g/ml) and screened by direct colony PCR and restriction digestion of plasmids. The sequences of inserts were verified. The overproduction of the ODC as inclusion bodies in the cytosol of *E. coli* was accomplished as described first for the bovine oxoglutarate-malate carrier (19), except that the host cells were *E. coli* CO214(DE3) (20). Control cultures with the empty vector were processed in parallel. Inclusion bodies were isolated, and ODC was purified by centrifugation and washing steps as described previously (19, 21).

Transport Assays—The recombinant protein in Sarkosyl was reconstituted into liposomes in the presence of substrates, as described previously (9). External substrate was removed from proteoliposomes on a Sephadex G-75 columns. The transport activity at 25 °C was determined by measuring the uptake (forward exchange) or the efflux (backward exchange) of [¹⁴C]oxoglutarate in exchange for unlabeled counter-substrates (22). For backward exchange measurements the proteoliposomes containing 1 mM internal oxoglutarate were prelabeled, after reconstitution, by carrier-mediated exchange equilibration by adding 10 μ M [¹⁴C]oxoglutarate (22). After 30 min, the residual external radioactivity was removed by passing the proteoliposomes through a column of Sephadex G-75. In forward exchange reactions, transport was started by adding [¹⁴C]oxoglutarate to the proteoliposomes and in the backward exchanges by adding nonradioactive substrate. In both cases, transport was stopped after 1 min (in the initial linear range of substrate exchange) by addition of 10 mM pyridoxal 5'-phosphate and 10 mM bathophenanthroline (the "inhibitor-stop" method (22)). In controls, the inhibitors were added at the beginning together with the external substrate. Finally, the external substrate was removed, and the radioactivity in the liposomes was measured (22). In forward exchange measurements, the experimental values were

corrected by subtracting control values, and the rate was calculated in millimoles/min/gram of protein. In the case of backward exchanges, the rate in Δ cpm/min was obtained from the decrease of internal radioactivity in 1 min. The reconstituted protein was assayed for other exchange activities by the inhibitor-stop method (22).

Other Methods—Proteins were analyzed by SDS-PAGE and stained with Coomassie blue dye. N-terminal sequencing was carried out as described previously (21). The amount of pure ODC was estimated by laser densitometry of stained samples (21). The amount of ODC incorporated into liposomes was measured as described previously (23) and varied between 29 and 37% of the protein added to the reconstitution mixture. Western blotting was carried out as described previously (24) with a rabbit antiserum raised against the bacterially expressed rat ODC protein.

RESULTS

Sequences of the Human and Rat ODCs—By screening data bases, nematode and fruit fly clones (R11.1 and AAF45544.1) were found with 36 and 38% of the residues of their encoded proteins identical, respectively, to the yeast ODC protein sequences. A human EST data base was interrogated with these sequences, and a clone of 286 bp (R29313) was identified that encoded a protein sequence that was 62 and 68% identical, respectively, to regions of the *C. elegans* and the *D. melanogaster* proteins.

The human EST was extended in the 5'- and 3'-directions by two PCR experiments. Among the products of the 5'-extension reaction, one band of about 1200 bp hybridized with primer 2F. Its sequence overlapped the EST. The 3'-extension product contained a single band of about 950 bp. Its sequence overlapped the 5'-extension. The final human cDNA sequence of 2024 nucleotides (Fig. 1) consisted of a 310-bp 5'-untranslated region, followed by an open reading frame of 900 bp, a 814-bp 3'-untranslated region containing a polyadenylation signal at nt 1974–1979 (25), and a poly(A)⁺ tail (see Fig. 1). The ATG codon at nt 311–313 is preceded by an in-frame stop codon 102-bp upstream and is likely to be the translational initiation codon. The N terminus of the human protein is at approximately the same position as in the rat, yeast, nematode, and *D. melanogaster* orthologs, confirming this view. The open reading frame encoded a polypeptide of 299 amino acids with a calculated isoelectric point of 9.6 and a molecular mass of 33,300 Da.

The cDNA for the rat ODC was cloned in a similar way. This sequence (GenBank™ accession number AJ289714) consists of 1456 nucleotides with a 99-bp 5'-untranslated region, followed by an open reading frame of 897 bp, and a 460-bp 3'-untranslated region, containing neither a polyadenylation signal nor a poly(A)⁺ tail. The open reading frame encoded a polypeptide of 298 amino acids with a molecular mass of 33,276. The human ODC is 82, 56, 55, 32, and 33% identical to the *R. norvegicus*, *C. elegans*, *D. melanogaster*, and *S. cerevisiae* ODC1 and ODC2 proteins, respectively.

Chromosomal Location of the Gene for Human ODC—The gene for human ODC was found on chromosome 14 at 14q11.2 by FISH experiments conducted on normal human metaphase chromosomes (Fig. 2). There was no evidence in this experiment for a second gene encoding an isoform, and no isoforms were detected in searches of data bases of human, nematode, and fruit fly sequences.

Expression of ODC in Various Tissues—The tissue distribution of mRNAs for the human and rat ODCs was studied by RT-PCR performed on total RNA populations, using primers and probes from regions of identity between the human and the rat nucleotide sequences. The ODC was detected in all tissues that were examined (Fig. 3A, panel a). The relatively weak signal from heart arises because less total RNA was employed than in other tissues, as the control demonstrates (see panel b in Fig. 3A). A similar pattern of expression was observed by Western blot analysis of rat mitochondria (see Fig. 3B, panel a).

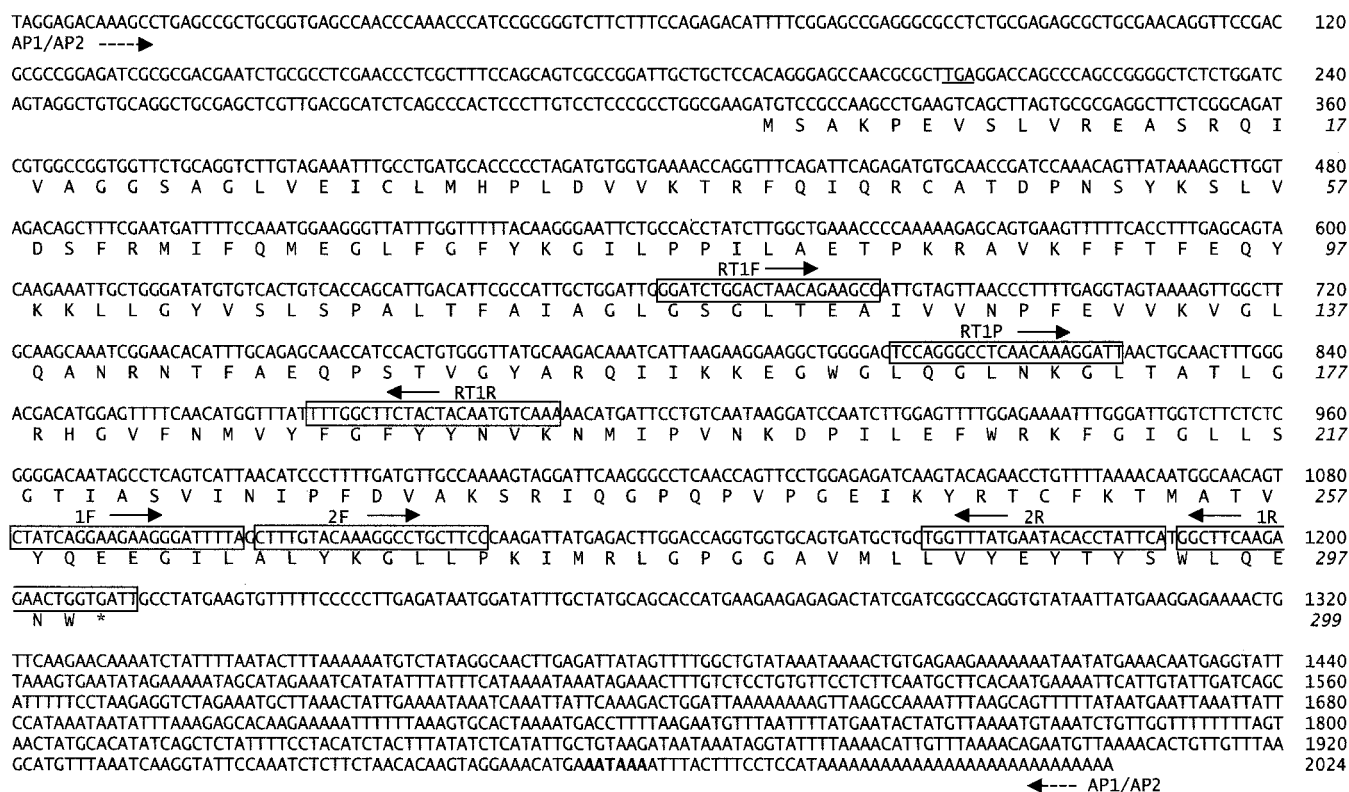


FIG. 1. Sequences of the cDNA for the human ODC and of the encoded protein. Amino acids are numbered from 1 to 299. The stop codon is denoted by an asterisk. The boldface sequence is a potential polyadenylation signal. The underlined sequence in the 5'-region is a stop codon in-frame with the initiator methionine. The partial cDNA sequence was extended in 3'- and 5'-directions with primers AP1 and AP2 and nested oligonucleotides 1F/2F or 1R/2R, respectively. Primers RT1F and RT1R and probe RT1P were employed in reverse transcription polymerase chain reaction experiments. Primer sequences are boxed; horizontal arrows pointing to the right or left indicate that the primers were synthesized as either the sequence shown or its complement, respectively.

Bacterial Overexpression of the Human ODC—The human ODC was overexpressed in *E. coli* C0214(DE3) (see Fig. 4, lane 4) in the form of inclusion bodies. The purified protein gave a single band by SDS-PAGE (Fig. 4, lane 5) with an apparent molecular mass of 32 kDa. The protein was not detected in bacteria harvested immediately before induction of expression (Fig. 4, lanes 1 and 2), nor in cells harvested after induction but lacking the coding sequence in the expression vector (lane 3). The N-terminal sequence (SAKPEVSLVR) of residues 1–10 of the purified protein was identical to that predicted for residues 2–11 of the human ODC (Fig. 1). About 35 mg of purified protein were obtained per liter of culture.

Functional Characterization of Human ODC—Proteoliposomes reconstituted with recombinant ODC catalyzed a counter-exchange of external [14 C]oxoglutarate for internal oxoglutarate with first order kinetics (rate constant 0.08 min^{-1}), isotopic equilibrium being approached exponentially (data not shown). The exchange reaction was inhibited completely by a mixture of pyridoxal 5'-phosphate and bathophenanthroline. In the absence of substrate in the proteoliposomes, or if the solubilized protein was boiled before the incorporation into liposomes, there was no uptake of labeled external substrate. Similarly, no oxoglutarate/oxoglutarate exchange was detected by reconstitution of Sarkosyl-solubilized material from bacterial cells either lacking the expression vector for ODC or harvested immediately before induction of expression. Furthermore, the proteoliposomes did not catalyze homoexchange activities for phosphate, carnitine, glutamate, aspartate, glutamine, ornithine, L-malate, ADP, and ATP (internal concentration, 10 mM; external concentration, 1 mM).

Substrate Specificity and Inhibitor Sensitivity—The substrate specificity of human ODC was investigated in greater

detail by measuring the uptake of [14 C]oxoglutarate into proteoliposomes that had been preloaded with a variety of substrates (Fig. 5A) or the efflux of [14 C]oxoglutarate from proteoliposomes in the presence of external nonradioactive substrates (Fig. 5B). High [14 C]oxoglutarate transport activities were observed when oxoglutarate, oxoadipate, glutarate, and adipate were used as counter-substrates on both sides of the liposomal membrane. High activities were also found with external pimelate and oxopimelate. To a lesser extent, internal pimelate, oxopimelate, and 2-aminopimelate, as well as both internal and external 2-aminoadipate, citrate, and oxaloacetate, also exchanged for labeled oxoglutarate. Low exchange, if any, was found with aspartate, fumarate, glutamate, glutathione, isocitrate, malate, maleate, malonate, oxalate, pyruvate, suberate, and succinate (Fig. 5, A and B).

The uptake of 0.2 mM [14 C]oxoglutarate by proteoliposomes containing 20 mM oxoglutarate (reaction time, 1 min) was inhibited almost completely by 2 mM pyridoxal 5'-phosphate (97%) and partly by 2 mM bathophenanthroline and 2 mM 2-cyanocinnamate (73 and 69% inhibition, respectively). Low concentrations of organic mercurials (10 μM) also inhibited markedly the ODC activity (*p*-chloromercuribenzenesulfonate, 58%; HgCl_2 , 64%; and *p*-hydroxymercuribenzoate, 47%). No significant inhibition was observed with 2 mM butylmalonate, phenylsuccinate, 1,2,3-benzenetricarboxylate, and *N*-ethylmaleimide (inhibitors of other characterized mitochondrial carriers) and 10 μM carboxyatractyloside (a powerful inhibitor of the ADP/ATP carrier).

Kinetic Characteristics of Human ODC—To obtain kinetic information about the [14 C]oxoglutarate/oxoglutarate exchange, the dependence of the exchange rate on substrate concentration was investigated at different concentrations of



FIG. 2. **Chromosomal location of the gene for the human ODC.** A human metaphase spread was hybridized *in situ* with a human ODC probe generated by PCR. The red probe is a 4.2-kb genomic fragment of human ODC. The arrow indicates the location of ODC at 14q11.2. A partial metaphase spread, from a different metaphase, is shown in the inset. Pseudocoloring and merging of images were performed with Adobe Photoshop™.

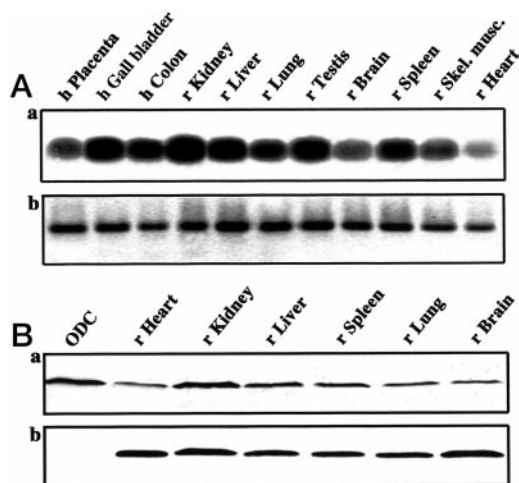


FIG. 3. **Expression of the ODC in various tissues.** A, analysis of total RNA from human (*h*) and rat (*r*) tissues. Panel *a*, hybridization with probe RT1P of the ODC cDNA fragments obtained by RT-PCR. panel *b*, ethidium bromide staining of the β -actin cDNA fragments obtained by RT-PCR. B, immunodetection of the ODC protein in mitochondria isolated from rat tissues. In panels *a* and *b*, mitochondria (150 μ g of protein) and recombinant rat ODC (75 ng) were exposed to antisera to ODC and subunit IV of the cytochrome *c* oxidase, respectively.

externally added [14 C]oxoglutarate (0.05–2 mM) at a constant internal concentration of 20 mM. The transport affinity (K_m) and the specific activity (V_{max}) values for oxoglutarate exchange at 25 °C, calculated from a standard double-reciprocal set of 35 experiments, were 0.22 ± 0.02 mM and 7.5 ± 1.2 mmol/min/g of protein, respectively. The activity was calculated by taking into account the amount of ODC recovered in the proteoliposomes after reconstitution.

The inhibition constants (K_i) of several externally added

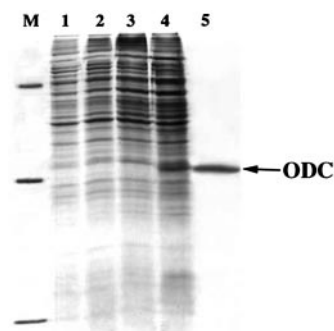


FIG. 4. **Overexpression of the human ODC in *E. coli*.** Proteins were separated by SDS-PAGE and stained with Coomassie Blue. Lane *M*, markers (bovine serum albumin, carbonic anhydrase, and cytochrome *c*); lanes 1–4, *E. coli* C0214(DE3) containing the expression vector, without (lanes 1 and 3) and with the coding sequence of ODC (lanes 2 and 4). Samples were taken at the time of induction (lanes 1 and 2) and 5 h later (lanes 3 and 4). The same number of bacteria was analyzed in each sample. Lane 5, purified ODC protein (1.2 μ g) originated from bacteria shown in lane 4.

substrates are summarized in Table I. All of them increased the apparent K_m without changing the V_{max} of [14 C]oxoglutarate/oxoglutarate exchange (not shown) acting as competitive inhibitors. In general, the oxoglutarate/oxoglutarate exchange was prevented by external addition of each of the substrates that are transported by human ODC (Fig. 5, A and B), and it was not affected by substrates of other mitochondrial carriers such as phosphate, ADP, ornithine, and carnitine. However, suberate, which is not transported by the ODC protein, inhibited the exchange activity significantly (Table I), suggesting that it binds to the substrate binding site of ODC without being transported.

DISCUSSION

The phylogenetic distance between *S. cerevisiae* and man frequently precludes the use of yeast sequences to identify human orthologs directly with certainty. The problem is even more severe when the protein in question is a member of a family with different functions but related sequences, as are the two isoforms of the yeast oxodicarboxylate carrier (9), which is a recently identified member of the family of mitochondrial transport proteins. Known family members are involved in the traffic of various substrates and metabolites across the inner membranes of the organelle. One possible solution, employed successfully in the cloning the rat dicarboxylate carrier (16), is to use the yeast sequence to identify orthologs in phylogenetically intermediate species where the genome sequence is known, such as *C. elegans* and *D. melanogaster*, and then to use these orthologs to identify potential orthologs in man. Thus, the yeast ODC isoforms were used to find clones in the *C. elegans* and *D. melanogaster* genomes, and they were used to identify a short related protein sequence in a human EST. This sequence proved to be part of the human ODC and provided the route to the complete human and rat ODC sequences. Both have the tripartite structure and the sequence motif that are characteristic of the mitochondrial carrier family (10–13).

The transport characteristics of the human ODC are similar to the yeast ODC isoforms. The main differences are the ability of the human protein to transport 2-aminoadipate, although rather poorly, and its incapacity to transport L-malate. The properties of the human ODC differ markedly from those of the oxoglutarate-malate carrier, which has greatest affinities for C4 and C5 oxodicarboxylates and dicarboxylates (19, 26, 27), whereas the human ODC prefers the C5–C7 homologs (Fig. 5 and Table I). In contrast to the bovine oxoglutarate-malate

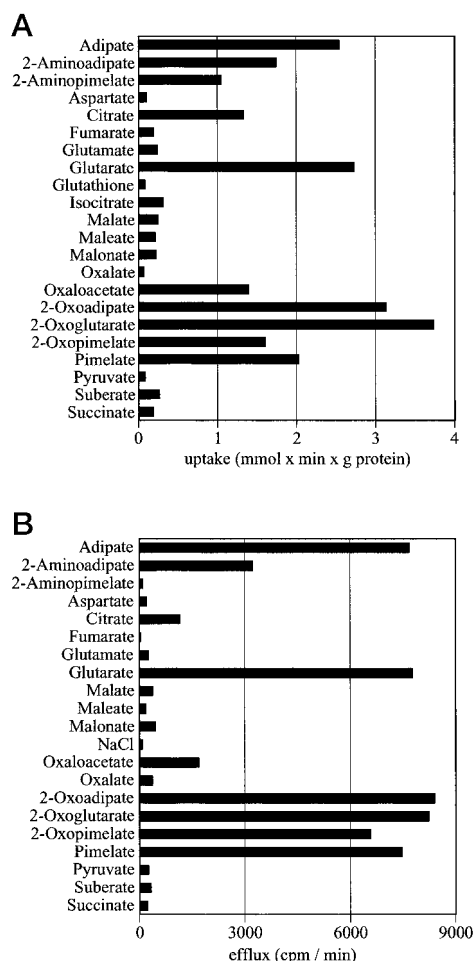


FIG. 5. **Substrate specificity of human ODC.** A, dependence of ODC activity on internal substrate. Proteoliposomes were preloaded internally with the indicated substrates (concentration, 20 mM). Transport was started by the addition of 0.2 mM [14 C]oxoglutarate and stopped after 1 min. B, dependence of ODC activity on external substrate. Proteoliposomes were preloaded internally with 1 mM oxoglutarate and then the internal substrate pool was labeled by carrier-mediated exchange equilibration. After removal of external substrate by Sephadex G-75 chromatography, the efflux of [14 C]oxoglutarate was started by adding the indicated substrates (concentration, 1 mM) and terminated after 1 min. At time 0 the intraliposomal radioactivity was 24,200 cpm and did not varied in the absence of external substrate for 30 min.

carrier, the human ODC does not transport malate, succinate, and maleate, but it does transport citrate, albeit at low efficiency. The low sequence identity between the ODC and oxoglutarate-malate carrier is also consistent with their different properties and functions.

The best substrates for the human ODC, both on the external and the internal membrane surfaces, are 2-oxoadipate and 2-oxoglutarate. Therefore, the physiological role of the human ODC is most likely to be to catalyze the uptake of 2-oxoadipate into the mitochondrial matrix in exchange for internal 2-oxoglutarate, thus performing a central role in catabolism of lysine, hydroxylysine, and tryptophan. Since ODC functions by a counter-exchange mechanism, the carrier-mediated uptake of 2-oxoadipate requires the efflux of a counter-substrate. On the basis of transport measurements, 2-oxoglutarate may serve as the counter-substrate of ODC for 2-oxoadipate. The efflux of 2-oxoglutarate is required by the lysine-oxoglutarate reductase, which, in the first step of lysine catabolism, converts lysine and 2-oxoglutarate into saccharopine. In agreement with its central role in cell metabolism, the human ODC is expressed in all tissues that were analyzed.

TABLE I

K_i values for substrates competing with [14 C]oxoglutarate uptake

The K_i values were calculated from double-reciprocal plots of the rate of [14 C]oxoglutarate/oxoglutarate exchange under variation of external oxoglutarate. Substrate concentrations were as follows: 20 mM internal 2-oxoglutarate and 0.05–2.0 mM external labeled 2-oxoglutarate. The competing substrates were added simultaneously with [14 C]oxoglutarate at the appropriate concentrations. The values are averages \pm S.D. of at least three experiments.

Substrate	K_i mM
2-Oxoadipate	0.11 \pm 0.01
Adipate	0.14 \pm 0.01
Pimelate	0.40 \pm 0.04
Glutarate	0.70 \pm 0.07
2-Oxopimelate	1.18 \pm 0.12
Suberate	6.6 \pm 0.8
Citrate	11.5 \pm 1.3
2-Amino adipate	12.7 \pm 1.5
Oxaloacetate	18.7 \pm 2.2
L-Malate	>25
2-Aminopimelate	>25

Another possible role for the human ODC may be to catalyze the uptake of 2-amino adipate into the mitochondrial matrix when this amino acid is not rapidly transaminated to 2-oxoadipate in the cytosol, for example after diets rich in amino acids that cause a decrease in 2-oxoglutarate content in the cytosol and consequently an inhibition of the 2-amino adipate aminotransferase. In this respect it is worth mentioning that transaminases interconverting oxoadipate and amino adipate are present both in the cytosol and inside the mitochondria (28, 29). The physiological role of ODC also suggests its possible involvement in 2-oxoadipate acidemia, which is accompanied by accumulation and excretion of large amounts of 2-oxoadipate, 2-amino adipate and 2-hydroxyadipate in urine, and by the clinical symptoms of mental retardation, hypotonia, motor and developmental delay, cerebellar ataxia, and learning disability (1–7). The molecular defect(s) responsible for this disease have not been characterized. Fibroblasts of patients with this condition are unable to oxidize 2-amino[1- 14 C]adipic and 2-oxo[1- 14 C]adipic acid to 14 CO $_2$ (2, 6) to any significant extent. Therefore, it was suggested that the disease may be due to defective 2-oxoadipate dehydrogenase, but no such defect has ever been demonstrated. The alternative possibility that defective ODC might provide the basis for this human metabolic disease can now be investigated.

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EXPRESSION, RECONSTITUTION, FUNCTIONAL CHARACTERIZATION,
TISSUE DISTRIBUTION, AND CHROMOSOMAL LOCATION**

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J. Biol. Chem. 2001, 276:8225-8230.

doi: 10.1074/jbc.M009607200 originally published online November 16, 2000

Access the most updated version of this article at doi: [10.1074/jbc.M009607200](https://doi.org/10.1074/jbc.M009607200)

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