Molecular cloning and characterization of ornithine decarboxylase cDNA of the nematode *Panagrellus redivivus*

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In a PCR with degenerate primers encoding highly conserved amino acids within ornithine decarboxylases (ODCs) of several organisms, a fragment of the ODC gene of the free-living nematode *Panagrellus redivivus* was isolated. Northern blot analysis revealed a single 1.7 kb transcript in a mixed-stage population of animals. From this RNA source, a cDNA library was constructed and screened with the PCR fragment. Several cDNA clones were isolated, one of which encodes the complete

435-amino-acid ODC enzyme with a calculated molecular mass of 47.1 kDa. The *P. redivivus* ODC possesses 126 of the 136 highly conserved amino acids in the enzymes from fungi, invertebrates and vertebrates. Functional amino acids are conserved, suggesting that the two active sites of the *P. redivivus* ODC are formed at the interface of a homodimer, as described for mammalian ODCs.

INTRODUCTION

Ornithine decarboxylase (ODC) is the first and key regulatory enzyme in the polyamine biosynthetic pathway [1,2]. ODC activity and polyamines play an indispensable role in normal cell growth and proliferation [3–6]. ODC gene expression and activity is rapidly and greatly promoted in response to growth factors and growth stimuli [7–9]. c-Myc protein, a product of the proto-oncogene *c-myc*, was demonstrated to be a potent transactivator of mammalian ODC gene expression at the level of transcription [10,11]. Furthermore, the cellular optimum of ODC activity is achieved by combined regulatory control mechanisms [1] at the level of ODC transcript stability [12,13], translational efficiency of mRNA [14–16] and post-translational modifications [17]. The rapid degradation of ODC is mediated by the action of the ODC-antizyme via the 26 S proteasome [1,18].

It is well established that eukaryotic ODC is enzymically active only as a homodimer [19] and is dependent on the cofactor pyridoxal 5'-phosphate (PLP) [8]. Poulin et al. [20] presented results obtained by studying the formation of adducts with PLP or the suicide inhibitor α -(difluoromethyl)ornithine (DFMO) suggesting that Lys-69 acts as the PLP binding site and Cys-360 as the major DFMO binding site. This implies that these amino acids, which are contained in conserved peptide motifs, represent key residues at the active site of the enzyme. Moreover, Tobias and Kahana [21] provided evidence that the active site of mouse ODC is formed at the interface between two monomers and involves residue Cys-360 from one subunit and Lys-69 from the other. This model rests on the finding that in transcription/translation experiments *in vitro* the individual ODC mutants Lys-69 $\rightarrow$ Ala and Cys-360 $\rightarrow$ Ala alone had no ODC activity. However, co-expression of both ODC mutants was partially able to restore ODC activity. Colemann et al. [22] expressed the recombinant mutant enzyme in *Escherichia coli* and confirmed that these residues play a key role in ODC activity. Furthermore, *in vitro* expression and subsequent measurement of ODC activity

revealed a substantial decrease in enzyme activity caused by the mutations Lys-69 $\rightarrow$ Ala, Lys-69 $\rightarrow$ Arg, Lys-115 $\rightarrow$ Arg, Lys-169 $\rightarrow$ Ala, Lys-169 $\rightarrow$ Arg, His-197 $\rightarrow$ Ala, Cys-369 $\rightarrow$ Ala and Cys-360 $\rightarrow$ Ser [23–26], indicating that these amino acid residues have an important function in ODC activity.

The primary structure of ODCs from several vertebrates and invertebrates, including *Caenorhabditis elegans*, has been deduced from cDNA sequences [27–29], Macrea and Coffino, EMBL data library Acc-no. U03059, 1993); however, there is no information available on the molecular genetics of ODC from parasitic nematodes. In order to establish a model nematode with similarities in overall structure and development to parasitic nematodes for studying polyamine metabolism in culture at the biochemical and molecular level, we investigated the free-living nematode *Panagrellus redivivus* as an experimental system. In the present study we describe the isolation of *P. redivivus* ODC genomic and cDNA clones and compare the complete deduced amino acid sequence with those of ODCs from other organisms.

EXPERIMENTAL

Biological material

A wild-type strain of *Panagrellus redivivus* was kindly provided by U. Wyss, Institute of Pathophysiology, University of Kiel, Germany. *P. redivivus* was maintained in 60 ml of sterile medium [clear top fraction of 4% Peptone (Merck 7202) and 2% Bacto-Liver (Difco 0133-01-09)] at 20 °C in 20 cm glass dishes. After 7–10 days, 20 ml of the culture was used to inoculate 40 ml of fresh medium.

Isolation of genomic DNA

A culture of *P. redivivus* was centrifuged for 5 min at 3500 g and 4 °C. The nematodes were washed twice in PBS (140 mM NaCl,

Abbreviations used: ODC, ornithine decarboxylase; PLP, pyridoxal 5'-phosphate; DFMO, α -(difluoromethyl)ornithine.

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under accession no. X82199 PRODC.

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20 mM KH_2PO_4 , pH 7.2). The genomic DNA was isolated essentially according to Jowett [30].

PCR

Degenerate inosine-containing primers (PCR grade), representing all possible codons of the amino acids, were purchased from Biometra, Göttingen, Germany. The 100 μl reaction volume contained in 1 $\times$ reaction buffer (20 mM Tris/HCl, pH 8.4, 50 mM KCl), 3 mM MgCl_2 , 3 μM sense and antisense primer, 100 ng of genomic DNA, 0.2 mM of each dNTP (Pharmacia), 0.05 % (v/v) detergent W1 and 2.5 units of *Taq* polymerase (Life Technologies). The cycle conditions were similar to those described by Averof and Akan [31]: (a) initial denaturation for 2 min at 95 °C, (b) five early cycles of 45 s at 95 °C, 30 s at 40 °C, 2.5 min ramp time and 2 min at 72 °C, (c) 30 late cycles of 45 s at 95 °C, 30 s at 50 °C and 2 min at 72 °C, (d) after final extension of 7 min at 72 °C the reaction was cooled down to 4 °C. Samples of 10 μl reaction products were analysed by agarose gel electrophoresis for the presence of an appropriate PCR product. PCR products were cloned into pUC18 via blunt-end ligation (Surecloning Kit; Pharmacia). Recombinant clones were identified by restriction digests.

RNA isolation

The PBS-washed *P. redivivus* were transferred on to a 25 μm -pore-size sieve in order to eliminate PBS. The worms were lysed in a mixture of phenol and guanidinium isothiocyanate (Trizol; Life Technologies) and total RNA was isolated according to the manufacturer's instructions. Poly(A)⁺-containing RNA was then purified from total RNA by one passage over an oligo(dT)-cellulose column (mRNA Purification Kit; Pharmacia) for Northern blot analysis and by two consecutive passages over oligo(dT)-cellulose columns for cDNA synthesis.

cDNA synthesis and cDNA library construction

A 3.5 μg portion of poly(A)⁺ RNA of a mixed-stage population of animals was reverse-transcribed and subsequently cloned in λ Zap XR vector using the cDNA Synthesis and cDNA Cloning Kit according to the instructions of Stratagene. At least 1.5×10^6 independent recombinant λ phages were cloned, with only 1 % of these lacking a *P. redivivus* cDNA insert. *In vivo* excision of pBluescript phagemids containing ODC cDNA inserts was performed as described in instructions from the Stratagene ZAP cDNA Cloning Kit.

Screening of phage library and hybridization

Screening of phage cDNA library was performed as described by Sambrook et al. [32]. DNA probes for hybridization were ³²P-labelled by random priming (Boehringer Mannheim) and hybridizations were performed using standard high-stringency conditions [32].

Northern blot analysis

A 6 μg sample of poly(A)⁺-enriched RNA and 30 μg of total RNA were separated by 1.0 %-agarose/formaldehyde gel electrophoresis [32] and subsequently transferred overnight in 10 $\times$ SSC on to a GeneScreen Plus membrane and treated according to the manufacturer's instructions. The Northern blot filter was pre-hybridized in the hybridization solution for GeneScreen Plus [5 $\times$ SSC, 1 % (w/v) SDS, 50 % formamide, 5 $\times$ Denhardt's, 100 μg of tRNA] for 2 h at 45 °C and hybridized for 19 h at the same temperature in the presence of 100 ng of ³²P-labelled DNA

at 1×10^6 c.p.m./ml. The filter was washed at 45 °C in 1 $\times$ SSC/1 % SDS for 5 min and then 2 $\times$ 20 min. The wet Southern blot and Northern blot filters were sealed and exposed to Kodak X-Omat S film at -70 °C.

DNA sequencing

DNA sequencing of both strands was performed by the dideoxy chain-termination method (Sequenase Sequencing Kit; USB/Amersham) using vector-specific and gene-specific oligonucleotide primers (Applied Biosystems). DNA and amino acid sequence analysis was carried out using DNASIS and PROSIS computer software (Pharmacia).

RESULTS

Identification of a genomic ODC PCR fragment

A multiple alignment of the ODC amino acid sequences of *Mus musculus* [27], *Xenopus laevis* [33], *Drosophila melanogaster* [29], *Trypanosoma brucei* [34], *Caenorhabditis elegans* (EMBL accession no. U03059) and *Saccharomyces cerevisiae* [35] revealed highly conserved boxes (see Figure 4), which were appropriate for the design of oligonucleotide primers for PCR. On the basis of conserved amino acids 64–71 (box I, including Lys-69, the PLP binding site), 108–115 (box II) and 315–325 (box IV) corresponding to the sequence of the mouse enzyme, degenerate inosine-containing oligonucleotide primers were designed and used in the PCR (Figure 1). A combination of box I or box II and box IV oligonucleotide primers resulted in 0.9 kb and 0.8 kb PCR products respectively. The DNA sequence of the 0.9 kb PCR product (clone 1677d) was determined and the deduced

Figure 1 Design of PCR primers based upon conserved ODC peptide motifs

Box I sense primer encompasses amino acids 64–71, box II sense primer encompasses amino acids 108–115 and box IV antisense primer encompasses amino acids 316–324 of mouse ODC. Since the ODC sequences of all other organisms have at position 108 the conserved amino acid isoleucine, the conservative substitution to a valine residue in the mouse ODC was ignored. All possible triplet codes are presented below the conserved peptide motifs. In order to reduce the complexity of the primer population, inosine was introduced in some positions instead of all four nucleotides. The diagram at the bottom illustrates the location of the conserved boxes within the ODC protein.

Figure 2 Northern blot analysis of *P. redivivus* ODC mRNA

Total cellular and poly(A)⁺ RNA of a mixed-stage population of *P. redivivus* were subjected to 1.0%-agarose/formaldehyde gel electrophoresis, transformed on to GeneScreen Plus membranes and hybridized with the radiolabelled genomic ODC PCR product (1677d) of *P. redivivus*. Lane 1, 30 µg of total RNA; lane 2, 6 µg of poly(A)⁺-enriched RNA; lane 3, short exposure of lane 2. Lanes 1 and 2 were exposed for 168 h and lane 3 for 16 h respectively. The mobilities of RNA size standards (RNA-Marker II; Boehringer Mannheim) are indicated on the left, and the arrow shows a single 1.7 kb ODC mRNA.

amino acid sequence examined. The high degree of sequence identity with the conserved amino acids of ODCs from several organisms confirms the molecular cloning of a part of the *P. redivivus* ODC gene.

Identification of a *P. redivivus* ODC transcript and isolation of cDNA clones

In Northern blot analysis, total RNA and poly(A)⁺ RNA from a mixed-stage population of *P. redivivus* were hybridized with the genomic 0.9 kb PCR product and washed under reduced stringency conditions. A single 1.7 kb transcript was very abundant in both samples, as shown in Figure 2.

In order to isolate cDNA clones corresponding to this transcript, a *P. redivivus* λ-Uni-ZAP cDNA library was constructed and the PCR product was used as a probe for screening 150000 recombinant phages. A total of 40 positive cDNA clones were identified, of which 14 were purified further. In a PCR, a combination of the vector-specific sense T7 primer and the gene-specific antisense primer 1 (underlined in Figure 3), which was deduced from the DNA sequence of the PCR product, was used to identify the cDNA clone with the maximum insert length. One of these clones, P-ODC 18, generated a PCR product of 1.1 kb in length. Since the distance between the conserved region of the ODC start codon and antisense primer 1 should be at least 1 kb, P-ODC 18 was further analysed since it was likely to be a nearly full-length ODC cDNA clone.

Analysis of ODC cDNA and amino acid sequence comparison

This cDNA clone was subjected to sequence analysis and the resulting DNA sequence and deduced amino acid sequence are shown in Figure 3. P-ODC 18 contains a 1617 bp cDNA insert, including an 18-nucleotide poly(A) track at the 3'-terminus. A polyadenylation signal (AATAA) is located 18 bp upstream of the poly(A). The DNA sequence of P-ODC 18 is identical to the corresponding sequence of genomic PCR clone 1677d, with the exception of two small 49 bp and 47 bp introns at positions 679

1	GA	AGA	CTT	GAC	ATT	CTT	CTC	CTT	TCA	TAA	TTT	CTA	CCC	GCG	CAA	GGT
48	CAC	TGT	CCA	CTT	GCT	GCT	TCG	GGT	ATT	ATC	CTC	CCG	TCT	GGT	CGA	CAG
96	AAA	CTT	CTT	GTC	GCC	CAA	AAT	CCC	GTC	CCT	GCG	GAA	ATC	CAC	ATT	CGC
144	CGT	COG	CAC	GGA	AAG	GCC	GTG	CTT	GAA	CAA	CGA	TAA	COG	TCC	GAA	GCC
192	TCC	TTG	GTG	ACG	TTT	GAA	AAT	ATG	ACC	CTC	ACC	GCG	TGT	GTT	GAC	TAC
240	TAC	GAG	ATC	ATC	GCT	GCC	ACG	AAG	GTT	GCC	GTC	TGT	CGC	AAT	GCC	ATC
288	GAC	AAC	AAA	ACC	GTC	GCA	ACG	GCT	ATT	GCT	GCA	ACC	AGG	ACT	GTA	AAT
336	GGC	AAC	GAT	GAC	CCT	TTT	GTC	GTG	ATG	AAT	GTG	AGC	ACC	ATC	ATG	GCT
384	AAG	GTC	ATT	CAA	TGG	CAA	CGA	ACG	ATG	CCA	CGA	GTG	GCT	TGC	TAT	
432	GCC	GTG	AAG	TGT	AAT	GAC	GAT	AAA	GTG	TTG	TTG	AGA	ACC	TTG	GCG	GAT
480	CTG	GCC	ATG	GCC	TTC	GAC	TGC	GCC	AAG	GCC	GAA	ATC	GAG	AAA	GTC	
528	ATT	GCG	CTG	GTT	GGA	CCG	GAA	AAG	ATT	GTC	TAC	GCG	AAT	CCG	TGC	AAG
576	ACC	CGC	GGA	TTC	ATT	GCC	CAT	GCT	GAG	GCC	GCT	GGA	GTT	AAG	CGA	ATG
624	ACG	TTC	GAT	AGC	GTT	GAG	GAG	TTG	ACC	AAG	ATT	ANG	CAA	AAC	CAT	GCC
672	GAT	CCG	AGC	CTC	ATC	CTC	COG	ATC	AGT	GTC	AGC	GAC	CCG	ACG	GCG	CAA
720	TGC	CAA	CTC	GGA	ATC	AAG	TTT	GCT	TGC	GAC	CCT	GAA	ACC	GTC	GCC	CCA
768	AAA	CTG	CTT	CGA	AAA	GCG	GCA	GAT	ATG	GGA	ATG	AAT	GTC	ATC	GGA	ATC
816	TCT	TTC	CAT	GTC	GGA	AGC	GCC	TGC	AAT	GAA	CCA	GCT	ACC	TTC	CGC	ACG
864	GCT	TTG	GAG	TAT	GCT	CGT	GGT	CTG	TTC	GAT	CTA	GGC	ATC	AGC	CTC	GCC
912	CTC	TCC	ATG	ACA	CTG	CTT	GAC	ATT	GGT	GCG	GGG	TTC	CCC	GCG	GTC	GAC
960	ACA	GCC	CAT	ATT	AGC	CTT	GAC	GCC	TGT	GCT	GCT	GTT	ATC	AAT	CCA	GCT
1008	CTC	GAG	GAA	CTC	TTC	CCG	CTT	GAT	AGT	TGC	CCG	GAT	GTT	GAA	GTC	ATC
1056	GCC	GAG	CCT	GCG	AGG	TAC	TTT	GCT	TGT	GCT	GCT	GTG	TCA	GTC	ACT	ACA
1104	AAT	GTC	ATT	GCC	AGT	GTC	AAA	GTC	CCG	GCT	AGC	CGA	ATC	ACT	GAA	AAA
1152	GCC	GAC	GAT	GTC	AAC	CGC	GAC	GGA	TAC	ATG	TAC	TAC	ATG	AAC	GAC	GGC
1200	GTC	TAT	GCT	TCT	TTC	AAC	TGC	AAG	TTG	TTC	GAT	CAC	TAT	CAA	CCT	CGT
1248	GGA	ATG	CCA	TTG	GCT	GAG	CAC	GAT	GCC	GAT	GAA	CCA	CGC	TTC	CCG	GTT
1296	TGC	GTT	TGG	GGA	CCA	ACT	TGT	GAC	GGT	TTG	GAC	CAA	GTA	GAG	GAA	TCT
1344	TCC	GTC	ATG	CCG	AGA	CTC	TAT	GAC	GGT	GAC	TGG	TTG	TAT	TAT	CCT	GAC
1392	ATG	GCG	GCC	TAC	ACA	TCG	GTT	GCC	GCC	AGC	ACC	TTC	AAC	GGT	TTT	GAT
1440	AAG	CCG	AAA	ACT	TAC	TAC	TTC	ATC	GAT	GAG	GCA	ACG	CTT	GGA	AGC	ATT
1488	GTT	CGC	AAA	GCA	GAT	TCT	GCT	CCT	CGC	GGT	TGA	CGA	CTG	CCT	GAT	GCG
1536	TCG	CCA	ACA	CCA	AGT	ATA	ATC	GAT	GAA	CGA	AAA	TCT	GTT	GAA	ATT	GAA
1584	AAT	AAA	ATG	ATA	TGT	TAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	A

Figure 3 Nucleotide and deduced amino acid sequences of *P. redivivus* ODC cDNA (1677d)

The single-letter symbol for each amino acid is given below the central nucleotide of its codon. The stop codon (TGA) is indicated by an asterisk. The potential polyadenylation signal is indicated by an open arrowhead. The boundaries of the 0.9 kb PCR product are marked by arrows and the locations of two small introns are indicated by filled arrowheads. The sequence of the gene-specific antisense primer 1, which was used to identify the nearly full-length cDNA clone, is underlined.

	MTLTGCVDDY	10	P.r.
	MISQF	3	C.e.
	MTTKSTPSSLSVNCVLAQTE	20	T.b.
	MSSTQVGNALSSSTTTLVDSNSTVTQKKQYKGETLHNLLELKN	47	S.c.
MSSFTKDEFDCHILDEGFTAKDILDQKINEVSSDDKDAFYVADLGDILKKHLR-WLKALPRVT		63	M.m.
MNSFSNDDFDFFLEEGFSARDIVEQKINEVSLSDDKDAFYVADFGDIVKKHVR-WFKALPRVT		63	X.l.
MAAATPEIQFYERELNIRRVIEECDLQRLDQ--ALNICDLSSVERN-VRLWQKLLPRIK		56	D.m.
EIIAGTKVAVCRNAI-DNKTIVATAIAATRTVNGND--PFVVMNVSTIMAK-VIQWQRTMPRVA		70	P.r.
EIIIGDNKIGVLPKQV-DQLQMCRDIAASKDLQENDS--SFMLVDLDKIIR-FQLWKRELPMIE		126	C.e.
KSMDIVVNDLSCRFLGFFENRDALCKKISMNTCDEGDPFVFADLGDIVRKHET-WKKCLPRVT		83	T.b.
NQDLLELLPHEQAHPKIVQALKARIGRINNETCDPGEENSFFICDLGEVKRLFN-WVKELPRIK		110	S.c.
→			
PFYAVKCN-DSRAIVSTLAAIGTGFDCASTEIQLVQGLGVPARVYIYANPCQVSIKYYAASN		126	M.m.
PFYAVKCN-DGKAIKVTLSILGAGFDCASTEIQLVQSIGVSPERIIYANPCQVSIKYYAASC		126	X.l.
PFYAVKCN-DDPMVRLLAQLGAGFDCASTEIKVGLVGFVSPERIIYANPCQVSHLEYAKEH		119	D.m.
PCYAVKCN-DDKVLRLTLADLGMGFDCASTEIKVIGL-VGPEKIVYANPCQVTRGFIAHAAA		132	P.r.
PFYAVKCN-TDL-VLIRILASLGCDFGFDCASTEIDIVMGTGVSAERIIYANPCQVTRGFIAHAMDR		126	C.e.
PFYAVKCN-DDWRVLGTLAALGTGFDCASTEIQVRGIGVPPKIIYANPCQVTRGFIAHAMDR		146	T.b.
PFYAVKCNFPDK-VLSLLAELGVNFDCASTEIDRVLSMNISPDRIYANPCQVTRGFIAHAMDR		173	S.c.
Δ			
GVQMMTFDSEIELMKVARAHPKAKLVLRITATDDSKAVCRLSVKFGATLKTSLRLLERA-KELNI		189	M.m.
GVEKMTFDSEVELMKVARNHPNAKLVLRITATDDSKAVCRLSVKFGATLKTSLRLLERA-KELNV		189	X.l.
QVSNGTVNDEFYVYKLHYPNSNLIVRFKSEAKEACPLGDKFGCDADVDAAALMLLAKSLEL		183	D.m.
GVKRMTFDSEVELTKIKQNHADPSLILRISVSPTAQCGIKFGCDPETVAPKLLRKAADMGM		196	P.r.
DVKMTFDNPEELLKIAKLHPNAEMILRIAVSDPTATCPLNLKFGADPIIAAPQLKTAEEGI		190	C.e.
GVDMTFDCELEKVAKTHPKAKMVLRISTDDSLARCRLSKVGAKEVDCRFLEQA-KKLN		209	T.b.
NVMKSTFDNVEELHKIKKFPESQLLRITATDDSTAQCLRLSTYGCENNVDLKAI-KELGL		236	S.c.
Δ			
DVIGVSFHVSGGCTDPDTFVQVSDARCVFDMAT-EVGFS-MHLLDIGGGFPGSEDTKLKFEI		251	M.m.
DIIGVSFHVSGGCTDPQTYVQVSDARCVFDMAT-ELGFN-MHLLDIGGGFPGSEDTKLKFEI		251	X.l.
KVTGTSFHVSGGCTSELQAYDRAIKKAKNLKFGA-LLGYD-MDFLDIGGGFPGSDVVK--FEKI		243	D.m.
NVIGISFHVSGGCTNEPATFRTALEYARGLFDLGI-SLGLS-MTLLDIGGGFPGVDTAHISLDAC		258	P.r.
NVVGISFHVSGGCTNDASAYRNALQHAKNLCEIGE-GLGFK-MDIIDMGGGFPGAHHNP--FEKI		252	C.e.
DVTGVSFHVSGGCTDASTFAQAISDSRFVDMGT-ELGFN-MHLLDIGGGFPGTRDAPLKFEI		271	T.b.
NLAGVSFHVSGGCTDSTLYKAVRDARTVFDKAANEYGLPPLKILDVGGGF-QFES----FKES		295	S.c.
Δ			
TSVINPALDKYFPDSGVR--IIAEPGRYYVASAFTLAVNIIAKKTVMWKEQPGSDDEDESNEQ-		312	M.m.
TSVINPALDKYFPADSGVK--IIAEPGRYYVASAFTLAVNIIAKKVMVNEQSGSDDEEDAANDK		313	X.l.
AESVNTSVQRHFFDE-RVH--IIAEPGRFFVAAACTLVCKIHAKREIRNEAGKLD-----		295	D.m.
AAVINPALDELFPDLSCPDVEVIAEPGRYFACAAVSYTTNVIASVKVPASRITEKADVDNR--		320	P.r.
AETIRDALDEFPPDTNKRLYR--EPGRFFAAGPFSLVANIIHATEVPASKITKDPKDCADH--		313	C.e.
AGVINNALEKHFFPDLKLT--IIAEPGRYYVASAFTLAVNIIAKKATPGVQTDVGAHAESNAQ-		332	T.b.
TAFLRLALEEFFPVGCGVD--IIAEPGRYFVATAFTLASHVIAKRKL-----SENEA-----		345	S.c.
←			
TFMYVNDGVYGSFNCILYDH--AHVKALLTKRKPDEKYYSS-----SIW		356	M.m.
TLMYVNDGVYGSFNCILFDH--AHVKPVLTKKPKDEKYYSS-----SIW		357	X.l.
TVMYVNDGVYGSFNCILYDHQ--VVIAEHLDNAESLPHLKS-----LIW		339	D.m.
GYMYVNDGVYGSFNCILFDHYQPRGMPLAEHDADEPRFPV-----CVW		364	P.r.
GYMYVINDGVYGSFNCILFDHAPIGSPLFDTRNEKFMST-----W		357	C.e.
SFMYVNDGVYGSFNCILYDH--AVVRPLPQREPINEKLYPS-----SVW		376	T.b.
--MIYVNDGVYGMNLCILFDHQEPHPRTLYHNLEFHYDDFESTAFDLSINKTRSEYPYKYSIW		407	S.c.
GPTCDGLDRIVERCNL-PEMHVGDWMLFENDGAYTVAAASTFNGF-ORPNIIYVMSRPMWQLMK		418	M.m.
GPTCDGLDRIVERFEL-PELQVEDWMLFENDGAYTVAAASTFNGF-QRPITYVMSRPHWQLMH		419	X.l.
GPSCDGLDKISEDLHL-PNLNRGDLGFRNDGAYTMPIASAFNGF-EFPKTYFQAI*		394	D.m.
GPTCDGLDQ-VEESSVMPRLYEGDWLYYPMGAYTVAAASTFNGF-DKPKTYFIDEATLGSIV		426	P.r.
GPTCDGLDVEDKLMMPK-MNVGEWLYYPMGAYTVAAASTFNGF-SKVPVPMYVMSSEMWESIR		420	C.e.
GPTCDGLDQIVERYYL-PEMQVGEWLLFEDMGAYTVVGTSSFNGF-QSPTIYFFSGLPDHVR		438	T.b.
GPTCDGLDCIAKEYYMKHDVIVGDWYFPPALGAYTVAAASTFNGFEQTADIVYIDSELD*		466	S.c.
Δ			
QIQSHGFPPEVEEQDJGTLPMSCAQESGMDRHPAACASARINV*		461	M.m.
DIKEHGILPEVP--DLSALHVSCAQESGMELAPAVCTAASINV*		460	X.l.
RKADSAPRG*		435	P.r.
DSTHV		424	C.e.
ELKSQKS*		445	T.b.

Figure 4 Multiple alignment of deduced ODC amino acid sequences of several organisms

The ODC amino acid sequences of *Mus musculus* (M. m.) [27], *Xenopus laevis* (X. l.) [33], *Drosophila melanogaster* (D. m.) [29], *Panagrellus redivivus* (P. r.), *Caenorhabditis elegans* (C. e.), (EMBL accession no. U03059), *Trypanosoma brucei* (T. b.) [34] and *Saccharomyces cerevisiae* (S. c.) [35] were aligned. If the amino acids are identical in the sequences of at least six of the seven organisms, it is defined as conserved. Highly conserved amino acids are in boldface type, conservative exchanges are underlined, a gap is indicated by the symbol ~ and the stop of translation is marked by an asterisk. The active-site-forming residues of the mouse ODC [26] are marked by arrowheads. The arrow indicates the conserved ODC peptide motifs which were used for the design of PCR primers.

and 1152 respectively of the cDNA. The splice junctions fit well with the consensus sequence of ODCs from other nematodes [36].

Between the ATG start codon at position 213 and the TGA stop codon at position 1519 there is a single open reading frame coding for a 435-amino-acid protein with a calculated molecular mass of 47.1 kDa. The *P. redivivus* ODC amino acid sequence was aligned with the ODC sequences of *M. musculus*, *X. laevis*, *T. brucei*, *D. melanogaster*, *C. elegans* and *S. cerevisiae*. Among these organisms, 136 (32%) amino acids are identical in six out of the seven sequences (Figure 4). The *P. redivivus* ODC possesses 126 of these 136 conserved amino acids. Furthermore, 47% of the amino acids are identical between *P. redivivus* ODC and *C. elegans* ODC, and there are sequence identities to intermediately conserved amino acids in the ODCs from the other species (compare *P. redivivus* ODC residues 158–184 with those of *D. melanogaster*). Gly-102 and the conserved Ile-366 of the mouse ODC are missing from the *P. redivivus* ODC. In comparison with vertebrates, there is a 10-amino-acid extension at the N-terminus of the *P. redivivus* enzyme. At the C-terminus the *P. redivivus* ODC lacks the 36-amino-acid tail of the vertebrate enzyme, which was shown to function in rapid protein degradation [37,38]. Ser-303 of the mouse ODC, which is proposed to be a phosphorylation site [27], is missing from the *P. redivivus* ODC amino acid sequence as well as from that of other invertebrates. The major DFMO binding site, Cys-360 of mouse ODC, and the PLP binding site, Lys-69 [20], are absolutely conserved in all species compared. Interestingly, Phe-64, in conserved box I, is substituted in *P. redivivus* by a cysteine.

DISCUSSION

A cDNA encoding *P. redivivus* ODC was isolated from a λ -Uni-ZAP cDNA library using a genomic PCR product amplified with primers based upon conserved amino acids of ODCs from several eukaryotic species. Since the 1617 bp P-ODC 18 cDNA represents nearly the full length of the 1.7 kb ODC transcript, the 5'-non-translated region can be calculated to be between 212 bp and 300 bp. At the 3'-end there is 99 bp non-translated region which includes a polyadenylation signal and a poly(A) tail. The cDNA contains a 1305 bp open reading frame encoding a polypeptide chain of 435 amino acids with a calculated molecular mass of 47.1 kDa. This is only slightly smaller than the 462-amino-acid ODC of the mouse, with a calculated molecular mass of 51.1 kDa. The composition of charged amino acid residues is nearly identical to that of the mouse ODC, which is reflected by the similar calculated isoelectric points of the *P. redivivus* and mouse ODCs of pH 4.82 and pH 5.07 respectively. A comparison of the ODC amino acid sequences between vertebrates and other eukaryotic organisms revealed 136 highly conserved amino acids, which are nearly entirely conserved in *P. redivivus* ODC, especially those residues which were shown to form the active site of the mouse ODC [20,24,26,39]. From these results we conclude that the cDNA clone P-ODC 18 encodes *P. redivivus* ODC.

Moreover, in light of the conservation of key amino acid residues in the *P. redivivus* ODC when compared with mammalian ODCs, we suggest that the nematode ODC is enzymically active as a homodimer. This conclusion is based upon the presence in the *P. redivivus* ODC of the conserved Gly-379 which was shown to play an essential function in dimer formation in the mouse ODC [39]. Furthermore, the conservation of the peptide motifs surrounding the major DFMO binding site Cys-360 and the PLP binding site Lys-69 [20] of mouse ODC support the conclusion that in *P. redivivus* ODC two active sites are formed at the

interface between the subunits and involve, according to the shared active site model for ODC [21], Cys-360 from one subunit and Lys-69 from the other. In comparison with vertebrate ODCs, the C-terminal PEST sequence, which was shown to play an important role in rapid protein degradation *in vivo* [37,38], is absent from the *P. redivivus* ODC. It might be deduced from this finding that *P. redivivus* ODC is much more stable than the mammalian enzyme, as reported for *T. brucei* ODC [37].

In this paper we report the introduction of *P. redivivus* as a model nematode for parasitic nematodes in order to explore the molecular genetics of ODC, the key enzyme in polyamine biosynthesis. Although it is not possible to demonstrate in the human and animal filarial parasites *Onchocerca volvulus* and *Dirofilaria immitis* any ODC enzyme activity [40], it would be informative to compare the gene structure and mRNA expression of ODC between a free-living nematode and a parasitic nematode. The results presented here might lead us to the molecular genetic analysis of the ODC gene in parasitic nematodes and to an understanding of the adaptation to parasitism in the case of ODC at the molecular level.

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