Molecular cloning of adenylate kinase from the human filarial parasite *Onchocerca volvulus*

Jolodar, A.[1*] **and Brattig, N. W.**[2]

[1]Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; [2]Tropical Medicine Section, Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany

[*]**Correspondence:** A. Jolodar, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran. E-mail: jolodara@scu.ac.ir

(Received 10 May 2008; revised version 29 Nov 2008; accepted 30 Nov 2008)

Summary

Adenylate kinases (ADK) are ubiquitous enzymes that contribute to the homeostasis of adenine nucleotides in living cells. In this study, the cloning of a cDNA encoding an adenylate kinase from the filaria *Onchocerca volvulus* has been described. Using PCR technique, a 281 bp cDNA fragment encoding part of an adenylate kinase was isolated from an *O. volvulus* cDNA library. Use of this fragment as a probe allowed the isolation of a larger cDNA clone through the searching the GenBank expressed sequence tag database. The full-length cDNA encodes 236 amino acid residues with a predicted molecular mass of 26.177 kDa. The deduced amino acid sequence exhibited 80% identity to the homologous adenylate kinase identified from *Caenorhabditis elegans*. Domain analysis of the resulting protein sequence was found to contain "adenylate kinase signature" motif which is highly conserved in all known ADKs. Multiple alignments showed that the N-terminal is well conserved, whereas the C-terminal is the most variable region.

Key words: Adenylate kinase, Filaria, *Onchocerca volvulus*

Introduction

Human onchocerciasis, known as river blindness, is a complex disease caused by the filarial parasite *Onchocerca volvulus*, a tissue-dwelling nematode which is transmitted by the bite of an infected *Simulium* black fly. An estimated 37 million people are infected and nearly 90 million persons are at risk of infection mainly in tropical Africa (WHO Expert Committee on Onchocerciasis, 1987; Basanez *et al*., 2006).

Adenylate kinase (ADK; EC 2.7.4.3) catalyses the reversible transfer of the phosphoryl group from ATP to AMP, releasing two molecules of ADP and is considered to contribute to the homeostasis of cellular adenine nucleotide composition (Hardie, 2003). In *Escherichia coli* by using temperature sensitive mutants, it has been found that the enzyme is essential for growth (Huss and Glaser, 1983). Adenylate kinases play an important role in the ATP-regenerating system required for eukaryotic ciliary or flagella movements (Pullen *et al*., 2004). Adenylate kinases have been found to be associated with different bacterial species as a virulence factor (Markaryan *et al*., 2001; Munier-Lehmann *et al*., 2003). For coping with energetic and synthetic challenges, parasites require high activities of ADK. This enzyme has been reported from various parasitic protozoa as *Trypanosoma cruzi* (Bouvier *et al*., 2006), *Plasmodium falciparum* (Ulschmid *et al*., 2004), *Leishmania donovani* (Villa *et al*., 2003) as well as some helminthes like *Schistosoma mansoni* (Cao *et al*., 1992) and *Clonorchis sinensis* (Yang *et al*., 2005).

The aim of this study was to clone a cDNA encoding an adenylate kinase from the filaria *O. volvulus* which was identified through expressed sequence tag (EST) strategy and homology search, to prepare for further functional study of this gene.

Materials and Methods

Cloning of *Ov*-ADK

In order to clone cDNAs encoding *Ov*-

ADK, PCR amplification was carried out on aliquots of the *O. volvulus* infected larval cDNA library, kindly provided by the *O. volvulus* genome project (Steven A. Williams, Smith College, Northampton, Mass.) (Williams *et al*., 2000) as template using a sequence-specific forward primer together with T7 primer with initial denaturation for 5 min at 95°C, followed by 35 cycles of 45 s at 94°C, 1 min at 59°C, 1 min at 72°C and finally, 7 min of incubation at 72°C in 50 µl PCR SuperMix (Life Technologies, Hamburg, Germany) containing 20 pmol of each primer, deoxynucleotides (each at 220 µM), 1.5 mM $MgCl_2$, 10 ng cDNA, and 1 U *Taq* polymerase. PCR products were ligated into the TOPO-TA vector (Invitrogen, Hamburg, Germany) and transformed into *E. coli* TOP10F´. Recombinant colonies were cultured on LB-agar supplemented with 100 µg/ml of ampicillin. Single colonies were picked up and cultured overnight in liquid LBA medium. A commercial kit (QIA mini prep, QIAGEN, Hilden, Germany) was used to prepare plasmid DNA.

DNA sequencing and sequence analysis

The amplified cDNA fragments were sequenced in both strands using a dideoxy termination method and run on an Applied Biosystems 373 DNA sequencer. The sequence was determined for both strands by using overlapping fragments. Sequence comparisons with GenBank database were done using the BLAST software (Altschul *et al*., 1990) from the NCBI site (http://www.ncbi.nlm.nih.gov). The CDD-Search software from the NCBI site was used to determine the conserved domains (Marchler-Bauer *et al*., 2003). Multiple sequence alignments were done using the CLUSTAL_W program (Thompson *et al*., 1994) and edited with the BOXSHADE software (http://www.ch.embnet.org/software/BOX_form.html). Molecular mass and isoelectric point was determined through the deduced protein sequence by analysis with the Compute pI/MW tool software available at the Expasy website (http://ca.expasy.org/tools/pi_tool.html).

Results

Cloning and characterization of *Ov*-ADK

In order to clone cDNA encoding ADK from *O. volvulus*, a sequence-specific forward primer was employed in conjunction with T7 primer using the pBlue script SK-phagamid cDNA library as template. DNA sequencing of the amplified product of 281 bp fragment confirmed that it encoded a putative ADK protein and was applied for searching the database using the NCBI tool. After database searching, a 640-bp clone (AI096315) was found revealing an open reading frame (ORF) contiguous with the previous cDNA fragment. The complete nucleotide sequence designed as *Ov*-ADK was assembled from the two overlapping cDNA clones. The contig of *Ov*-ADK contained a cDNA of 767 bp with a single open reading frame of 708 bp which was terminated by TAG stop codon at position 760. The clone encodes a polypeptide of 236 amino acids with a calculated molecular mass of 26.177 kDa and theoretical pI of 5.27. The initiation codon was assigned by homology to known ADK sequences.

Identification of the *Ov*-ADK

Comparison with the sequences contained in the GenBank database revealed strong similarity with the ADK molecules described in various organisms. The N-terminal region is well conserved, whereas the C-terminal region is the most variable.

In Fig. 1, the sequence is aligned with eukaryotic ADKs from *Caenorhabditis elegans* (NM_077483), *Schistosoma japonicum* (AY274582), human (Van Rompay *et al*., 1999), pig (Heil *et al*., 1974) and chicken (Kishi *et al*., 1986). The highest level of identity was scored with a *C. elegans* (81%) ADK1, followed by *S. japonicum* (62%) ADK. A sequence analysis of *O. volvulus* ADK also shows 58% identity to ADK from chicken, *Bos taurus* and *Gallus gallus* and also 57% to human ADK. Domain analysis of *Ov*-ADK by the CDD-Search (Conserved Domain Database) showed e-value of 5.1e-79 with conserved domain of eukaryotic adenylate kinase (pfamcd01428) between amino acid residues

24 to 181. This region is conserved and "adenylate kinase signature" motif 102[FLIDGYPREVKQ]113, which is highly conserved in all known ADKs is present within this region (http://motif.genome.jp/). Three functional domains have been described in the primary structure of nucleoside monophosphate kinases: the nucleoside triphosphate binding glycine-rich region, the nucleoside monophosphate binding site, and the lid domain that closes over the substrate upon binding (Schultz, 1987; Fukami-Kobayashi *et al*., 1996). The alignment showed a highly conserved glycine-rich region at amino acid residues 26 to 34 corresponding to the binding site of the phosphate donor. Amino acid residues 49-79 are similar to the nucleoside monophosphate binding site of several other nucleoside monophosphate kinases. The lid domain contains many of the catalytically important residues and was present in *Ov*-ADK at amino acid residues 140-153. An analysis using the signal peptide software indicated that *Ov*-ADK enzyme is soluble and did not possess a signal peptide domain structure.

Discussion

The amino acid sequence of *Ov*-ADK is compared in Fig. 1 to adenylate kinase from several organisms including AKI, the pig heart cytosolic enzyme, whose three-dimensional structure has been determined (Heil *et al*., 1974). Based on the phylogenetic tree, ADK was divided into two main branches, the short (AK1 and AK5) and long (AK2, AK3, AK4 UMP/CMP kinase) enzymes subclasses. It has been shown that the long enzymes are dependent on the length of the lid domain (Fukami-Kobayashi *et al*., 1996). Adenylate kinase of *O. volvulus* is shorter than pig AK1 at the N- (11 residues) and C-terminus (30 residues). However, sequence

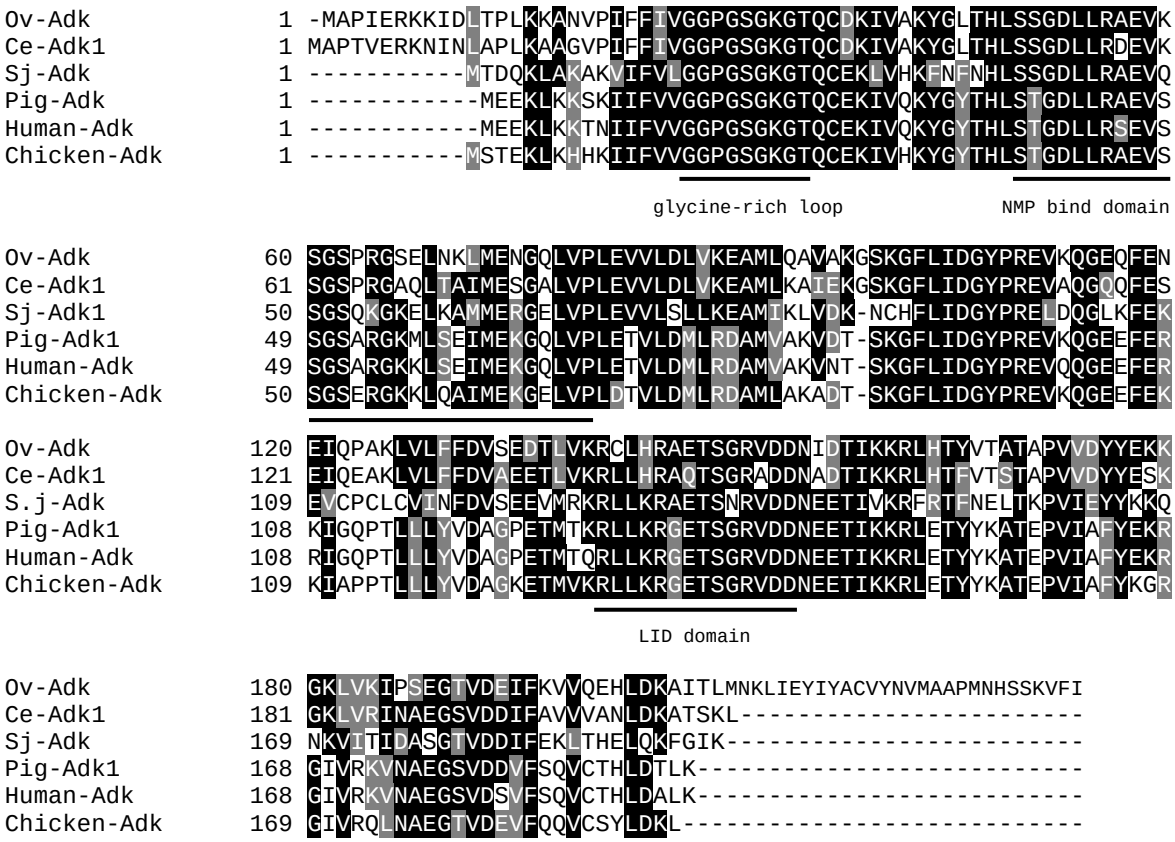

Fig. 1: Multiple sequence alignment of adenylate kinase from *O. volvulus* with other sources of adenylate kinase from *Caenorhabditis elegans* (NM_077483), *S. japonicum* (AY274582), human (NM_000476), pig (P00571) and chicken (M12153). The positions of the glycin-rich loop, NMP bind domain and LIM domain are underlined. Shading indicates identity (black) or conservative substitutions (grey) relative to *O. volvulus*

comparisons of the adenylate kinases showed that *Ov*-ADK has a similar size in the lid domain region. Therefore, it is possible to speculate that *Ov*-ADK is a member of the short enzyme subclasses.

Multiple sequence analysis shows the same regions of homology, where many residues are conserved between various adenylate kinases. They all show preserved regions of homology which are situated around residues 25-36, 46-58, 99-111 and 144-154, taking the position numbers from the *Ov*-ADK sequence. These stretches of homology can be assigned to various structural features of the three-dimensional structure, which together are believed to form the scaffold for the AMP and MgNTP nucleotide binding sites. All the residues in N-terminus part of NMP bind domain, that have been implicated to be involved in nucleotide binding are conserved except threonine 39, which in *Ov*-ADK is replaced by serine (residue 50). Since both residues are polar and uncharged with a hydroxyl group, it is conceivable that the side chain of serine performs the same function and it would be interesting to test, what effect, if any, the replacement of serine by threonine in *O. volvulus* adenylate kinase might have.

Acknowledgement

This work was supported by a fellowship (A. J.) from the Alexander von Humboldt Foundation, Bonn, Germany.

References

Altschul, SF; Gish, W; Miller, W; Myers, EW and Lipman, DJ (1990). Basic local alignment search tool. J. Mol. Biol., 215: 403-410.

Basanez, MG; Pion, SD; Churcher, TS; Breitling, LP; Little, MP and Boussinesq, M (2006). River blindness: a success story under threat? PLoS Med., 3: e371.

Bouvier, LA; Miranda, MR; Ganepa, GE; Alves, MJM and Pereira, CA (2006). An expanded adenylate kinase gene family in the protozoan parasite *Trypanosoma cruzi*. Biochem. Biophys. Acta. 1760: 913-921.

Cao, M; Akridge, R; Weston, D; Kemp, WM and Doughty, BL (1992). *Schistosoma mansoni*: cloning and sequencing of a gene for adenylate kinase. Exp. Parasitol., 74: 357-359.

Fukami-Kobayashi, K; Nosaka, M; Nakazawa, A and Go, M (1996). Ancient divergence of long and short isoforms of adenylate kinase: molecular evolution of the nucleoside monophosphate kinase family. FEBS Lett., 385: 214-220.

Hardie, DG (2003). Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. Endocrinology. 144: 5179-5183.

Heil, A; Müller, G; Noda, L; Pinder, T; Schirmer, H; Schirmer, I and von Zabern, I (1974). The amino-acid sequence of sarcine adenylate kinase from skeletal muscle. Eur. J. Biochem., 43: 131-144.

Huss, RJ and Glaser, M (1983). Identification and purification of an adenylate kinase-associated protein that influences the thermolability of adenylate kinase from a temperature-sensitive ADK mutant of *Escherichia coli*. J. Biol. Chem., 258: 13370-13376.

Kishi, F; Maruyama, M; Tanizawa, Y and Nakazawa, A (1986). Isolation and characterization of cDNA for chicken muscle adenylate kinase. J. Biol. Chem., 26: 2942-2945.

Marchler-Bauer, A; Anderson, JB; DeWeese-Scott, C; Fedorova, ND; Geer, LY; He, S; Hurwitz, DI; Jackson, JD; Jacobs, AR; Lanczycki, CJ; Liebert, CA; Liu, C; Madej, T; Marchler, GH; Mazumder, R; Nikolskaya, AN; Panchenko, AR; Rao, BS; Shoemaker, BA; Simonyan, V; Song, JS; Thiessen, RA; Vasudevan, S; Wang, Y; Yamashita, RA; Yin, JJ and Bryant, SH (2003). CDD: a curated Entrez database of conserved domain alignments. Nucleic Acids Res., 31: 383-387.

Markaryan, A; Zaborina, O; Punj, V and Chakrabarty, AM (2001). Adenylate kinase as a virulence factor of *Pseudomonas aeruginosa*. J. Bacteriol., 183: 3345-3352.

Munier-Lehmann, H; Chenal-Francisque, V; Ionescu, M; Christova, P; Foulon, J; Carniel, E and Barzu, O (2003). Relationship between bacterial virulence and nucleotide metabolism: a mutation in the adenylate kinase gene renders *Yersinia pestis* avirulent. Biochem. J., 373: 515-522.

Pullen, TJ; Ginger, ML; Gaskell, SJ and Gull, K (2004). Protein targeting of an unusual, evolutionarily conserved adenylate kinase to a eukaryotic flagellum. Mol. Biol. Cell. 15: 3257-3265.

Schultz, GE (1987). Structural and functional relationships in the adenylate kinase family. Cold Spring Harbor Symposium on Quantitative Biology. 52: 429-439.

Thompson, JD; Higgins, DG and Gibson, TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22: 4673-4680.

Ulschmid, JK; Rahlfs, S; Schirmer, RH and Becker, K (2004). Adenylate kinase and GTP:AMP phosphotransferase of the malarial parasite *Plasmodium falciparum*. Central players in cellular energy metabolism. Mol. Biochem. Parasitol., 136: 211-220.

Van Rompay, AR; Johansson, M and Karlsson, A (1999). Identification of a novel human adenylate kinase cDNA cloning, expression analysis, chromosome localization and characterization of the recombinant protein. Eur. J. Biochem., 261: 509-516.

Villa, H; Perez-Pertejo, Y; Garcia-Estrada, C; Reguera, RM; Requena, JM; Tekwani, BL; Balana-Fouce, R and Ordonez, D (2003). Molecular and functional characterization of adenylate kinase 2 gene from *Leishmania donovani*. Eur. J. Biochem., 270: 4339-4347.

WHO Expert Committee on Onchocerciasis, Third report. World Health Organization (1987). Tech. Rep. Ser., 752: 1-167.

Williams, SA; Laney, SJ; Lizotte-Waniewski, M; Bierwert, LA and Unnasch, TR (2000). The river blindness genome project. Trends Parasitol., 18: 86-90.

Yang, G; Yu, X; Wu, Z; Xu, J; Song, L; Zhang, H; Hu, X; Zheng, N; Guo, L; Xu, J; Dai, J; Ji, C; Gu, S and Ying, K (2005). Molecular cloning and characterization of a novel adenylate kinase 3 gene from *Clonorchis sinensis*. Parasitol. Res., 95: 406-412.