CLONING OF A MITOCHONDRIAL VOLTAGE-DEPENDENT ANION CHANNEL FROM THE SHARK RECTAL GLAND

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Voltage-dependent anion channels (VDAC) are small pore-forming channels found in the outer mitochondrial membrane of eukaryotic organisms. These channels are formed by a single 30-kDa protein, consisting of 1 alpha helix and 13 beta strands (Song et al., J. Biol. Chem., 273: 24406-13,1998). VDACs conduct adenine nucleotides, NADH, and cytochrome c and are associated with the adenine nucleotide translocator at the contact points between the inner and outer mitochondrial membranes (Sampson et al., Genomics, 33:283-288,1996, Xu et al., J. Membr. Biol.170:89-102,1999). Under the influence of a transmembrane voltage, reconstituted VDACs undergo gating from a high conducting anion-selective "open" state to lower current-conducting states. These lower states are referred to as "closed" as they are cation-selective and impermeant for nucleotide phosphates (Elkeles et al., J. Biol. Chem.,272: 6252-6260: 1997).

Voltage-gating of VDAC is regulated by physiological concentrations of beta-NADH, suggesting a possible mechanism for the effects of glycolysis on oxidative phosphorylation. Beta-NADH reduces the permeability for ADP in a concentration-dependent manner, while alpha-NADH and beta-NAD+ do not. In the presence of NADH, the opening and closing of this channel is more sensitive to changes in membrane potential and is better able to respond to changes in metabolic conditions. This effect was observed in both human and two fungal forms of VDAC, indicating a highly conserved regulatory mechanism (Zizi et al., J. Biol. Chem., 269: 1614-1616, 1994, Lee et al., J. Biol. Chem., 269: 3074-3080, 1994). In addition, VDACs bind several kinases important in intermediary metabolism, including isoforms of hexokinase and glycerol kinase. VDACs are thought to provide the kinases with preferential access to ATP derived from oxidative phosphorylation (Adams et al., Biochem. Med. Metab. Biol., 45:271-291, 1991). A recent study demonstrated the absence of detectable VDAC protein by Western blotting in a muscle sample from a child with mitochondrial myopathy, suggesting a possible phenotype for human VDAC mutations (Huizung et al., Lancet, 344: 762, 1994).

Three isoforms of VDAC have been identified in wheat, potato, human and mouse. Electrophysiological studies demonstrate differences in the channel properties of these isoforms. VDAC1 is the prototypic version whose properties are highly conserved among other species. VDAC2 also has normal gating activity but may exist in 2 forms, one with a lower conductance and selectivity. VDAC3 can form a channel in planar phospholipid membranes, but it does not

insert readily into membranes and generally does not gate well even at high membrane potentials (up to 80 mV) (Xu X. et al., J Membr. Biol. 170: 89-102, 1999).

A shark rectal gland Expressed Sequence Tag (EST) clone from the MDIBL Marine DNA sequencing center (clone number 12) had high homology to frog VDAC. The complete sequence of the clone revealed an open reading frame encoding for 283 amino acids with 80% identity to Frog VDAC2 and 77% identity to Human VDAC2. The obtained sequence includes the eukaryotic porin signature: [YH]-x(2)-D-[SPCAD]-x-[STA]-x(3)-[TAG]-[KR]-[LIVMF]-[DNSTA]-[DNS]-x(4)-[GSTAN]-[LIVMA]-x-[LIVMY].

Figure 1 shows the amino acid alignment between putative shark VDAC and human VDAC2.

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            1                                                        60
 Shark_VDAC MS...........VPPSYADLGKSARDLFNKGYGFGLVKLELKTKSSSGVEFTTSGSSNT
Human_VDAC2 MATHGQTCARPMCIPPSYADLGKAARDIFNKGFGFGLVKLDVKTKSCSGVEFSTSGSSNT

            61                                                       120
 Shark_VDAC DTGKATGSLETKYKLKEYGLTFTEKWNTDNNLATEITIEDQLAKGLKLTFDTTFVPNTGK
Human_VDAC2 DTGKVTGTLETKYKWCEYGLTFTEKWNTDNTLGTEIAIEDQICQGLKLTFDTTFSPNTGK

            121                                                      180
 Shark_VDAC KSGKLKTAYKRDYVNLGCDIDFDFAGPTIHSMAVFGYEGWLVGHQMAFDTAKSKLAQNNF
Human_VDAC2 KSGKIKSSYKRECINLGCDVDFDFAGPAIHGSAVFGYEGWLAGYQMTFDSAKSKLTRNNF

            181                                                      240
 Shark_VDAC SLGYKAGDFQLHTHVNDGAEFGGSIYQKVNDKVETAVNLAWTAGSNNTRFGIAAKYQIDS
Human_VDAC2 AVGYRTGDFQLHTNVNDGTEFGGSIYQKVCEDLDTSVNLAWTSGTNCTRFGIAAKYQLDP

            241                                                  294
 Shark_VDAC DAYVSAKVNNSSLIGVGYTHTLRPGVKLTLSGLIDGKNFHAGGHKVGMGFELEA
Human_VDAC2 TASISAKVNNSSLIGVGYTQTLRPGVKLTLSALVDGKSINAGGHKVGLALELEA
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Figure 1. Amino acid alignment of human and shark VDAC2. Non-identical amino acids are in bold.

The shark rectal gland (SRG) is an epithelial organ with high rates of ion transport. The gland is a major consumer of metabolic energy with the highest known Na/K-ATPase activity and high rates of oxygen consumption. Since VDACs are essential for ATP formation in the mitochondria, this particular protein may be a critical anion channel involved in energy production in the SRG.

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