Indications of a Common Folding Pattern for VDAC Channels from All Sources

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Previous research on the mitochondrial channel VDAC from the yeast *S. cerevisiae* had identified protein strands forming the wall of VDAC's aqueous pore. Here we report the results of analyzing the primary sequences of VDAC from various sources to see if the transmembrane folding pattern identified from this yeast is conserved for VDAC of different species. We analyzed the primary sequences of VDAC from higher plants, fungi, invertebrates, and vertebrates and found that all have a very similar "β-pattern" profile with 12–15 peaks indicating potential sided beta strands that are candidates for protein strands forming the wall of the aqueous pore. All these VDAC sequences can be put into the 13 transmembrane strand folding pattern previously identified for yeast VDAC. These folding patterns agree with available experimental data: both electrophysiological and protease digestion data. Although the primary sequences of VDAC from very diverse organisms show low homology, sequence similarity in the proposed corresponding 13 transmembrane strands is substantial. Competing proposals utilizing 16 transmembrane β strands are in conflict with electrophysiological experimental observations and violate the constraints on such strands, such as no charged amino acids facing the phospholipid membrane and sufficient number of residues to span the membrane.

INTRODUCTION

VDAC is a large channel-forming protein located on the mitochondrial outer membrane. The electrophysiological properties, including single-channel conductance, anion selectivity, and voltage dependence are highly conserved for the VDAC isolated from very different organisms (Colombini, 1989) which indicates that they have similar three-dimensional structures. The experimental results which have been collected point to the existence of an α-helix and 12 β-strand folding pattern in VDAC isolated from the yeast *S. cerevisiae* (Blachly-Dyson *et al.*, 1990; Peng *et al.*, 1992; Thomas, *et al.*, 1993). An alternating hydrophobic and hydrophilic pattern is required to form a trans-

membrane β-strand lining the wall of a simple single-walled aqueous pore. To find out if this folding pattern is conserved with evolution, we analyzed the primary sequences of VDAC from a vertebrate (human sequence 1), an invertebrate (the worm *C. elegans*), fungi (*Neurospora crassa, Saccharomyces cerevisiae, and Dictyostelium discoideum*) and plants (wheat, maize, pea, and potato) in terms of potential transmembrane β-strands. We tried to identify the strands corresponding to those identified to be transmembrane in yeast VDAC. We also examined how our folding patterns differed from those based on 16 strands (Rauch and Moran, 1994) and how well each agreed with experimental results.

MATERIALS AND METHODS

All the VDAC sequences were obtained from the Genetics Computer Group database. The accession

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numbers are as follows: human sequence 1, L06132; *C. elegans,* M75857; *N. crassa,* X05824; *S. cerevisiae,* X02324; *D. discoideum,* M96668; maize, S34146; pea, S36454; potato, X80388; wheat, X77733. In the rest of the manuscript these will be referred to as: human 1, worm, *Neurospora,* yeast, *Dictyostelium,* maize, pea, potato, and wheat.

The β-pattern profile was generated as first described by Blachly-Dyson *et al.* (1989). The hydropathy value (Kyte and Doolittle, 1982) of each group of 10 adjacent amino acids was combined as follows:

$$\sum_{i=1}^{10} (-1)^{i+1} v(i)$$

Where $v(i)$ is the hydropathy value of the ith amino acid. The absolute value of these sums was plotted against the number of the first amino acid in the summation. The higher the peak, the better the alternating polar/nonpolar pattern.

The criteria for choosing a sequence of ten amino acids as a transmembrane β-strand in the proposed folding patterns are as follows: (1) It has a good alternating hydrophobic and hydrophilic pattern. (2) There are no prolines in the sequence except at the ends. (3) There are no adjacent charged amino acids except at the ends. (4) There are no charged amino acids facing the membrane in the folding pattern. (5) It has good sequence homology to the corresponding transmembrane β-strand for yeast VDAC. (6) The folding pattern is consistent with experimental observations. (7) The folding pattern is similar to that of VDAC identified from yeast.

When counting the total net charges inside the channel, only those charged amino acids which are proposed to be inside the channel and face the interior of the channel are counted and given a value of either +1 or −1 as appropriate. The charged amino acids which are at the very end of the strand are given a value of +0.5 or −0.5 as appropriate.

RESULTS

The β-pattern of VDAC Is Well Conserved for All Species

The "β-pattern" was used as the first criterion to identify transmembrane β strands. This was calculated as indicated in Materials and Methods. As expected, this pattern is most similar for closely related species

(Fig. 1). Among the plants, VDAC sequences from the same class but different order (pea and potato vs. maize and wheat) have virtually identical patterns (Fig. 1A). One major difference, an extra peak, is seen in the pattern for wheat VDAC (arrow). The β-pattern of human 1 VDAC is also very similar to that of a partial VDAC sequence from worm (*C. elegans*) (Fig. 1B). These are in different phyla. Note that while the peak shapes and intensities vary somewhat, the location of the peaks in the sequence is virtually the same.

The β-pattern is also well conserved among VDAC from different kingdoms (Fig. 2). The correspondence between yeast and human VDAC is most direct (Fig. 2A). Regions on the human VDAC sequence (indicated by the 12 dips in the line above the β pattern) were identified which corresponded to the regions in yeast shown to form the aqueous pore. There are strong peaks in the β pattern of yeast that were shown by site-directed mutations *not* to form
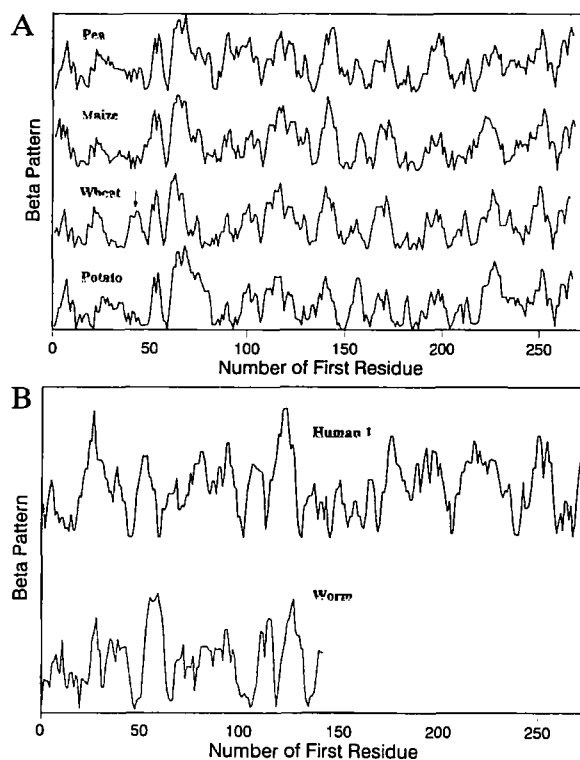

Fig. 1. Indications of a conserved secondary structure of VDAC isolated from species within the same kingdom. A The β-patterns (see materials and Methods) of VDAC sequences from wheat, maize, pea, and potato are illustrated. The extra peak in the wheat VDAC sequence is denoted by the arrow. B. The β-pattern of a human VDAC sequence (HVDAC1) is compared to a partial worm VDAC sequence.
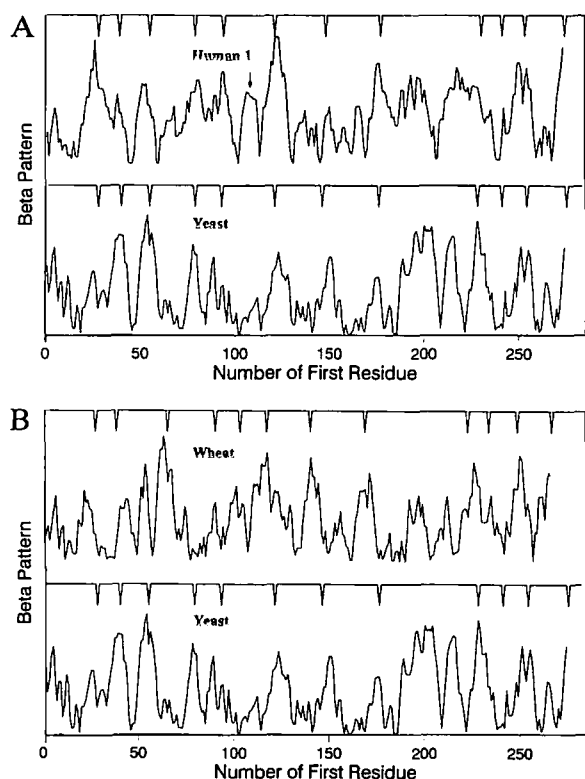
Fig. 2. Comparisons of the β-patterns of the VDAC sequences of yeast and human (A) and yeast and wheat (B). The small dips above the patterns point to the number of the beginning amino acid of each of the 12 transmembrane β-strands in the proposed folding patterns.

parts of the wall of the pore (Blachly-Dyson *et al.*, 1990). The corresponding regions in the human sequence were also presumed to form surface domains. Thus, only 12 transmembrane β strands are proposed for human 1 VDAC.

In the case of wheat, the β pattern shows substantial differences from that of yeast (Fig. 2B). There appears to be an additional peak around position 50 and a corresponding missing region around position 100. The assignments were made with the aid of the primary sequence in determining the corresponding region. Thus, with one segment insertion and one segment deletion, one can account for the bulk of the difference between yeast and wheat VDAC. Note that evidence for the one insertion and one deletion can be seen by comparing the two primary sequences (Fig. 3). Thus, once again the yeast transmembrane folding pattern can be found in wheat VDAC. Similar results were obtained with the sequence of *Dictyostelium* VDAC (not shown). However, the *Dictyostelium*

VDAC sequence is by far the most different of all available sequences and was the hardest to work with.

All VDAC Sequences Can Be Cast into a Very Similar Folding Pattern

After identifying the corresponding transmembrane strands to those in yeast VDAC, it is instructive to place them into a transmembrane folding pattern. In addition to the 12 β strands, the N-terminus is presumed to form a transmembrane α helix as is the case with yeast VDAC (Fig. 4A). The VDAC sequences of human 1 (B), *Neurospora* (C), *Dictyostelium* (D), and maize (E) are shown. There are a number of features that the patterns share. The distribution of polar and nonpolar residues in the region of the proposed α helix is such that one side of the helix is polar and the other is nonpolar as expected for a transmembrane strand forming the wall of the pore. There are no charged residues on alternate rows in the β strand portion as these would face the phospholipid tails (the only exceptions are at the very end of the strand where these residues would either face the surface or be the beginning of a turn). Residues with high potential of turn conformations, such as prolines, tend to be at the end of the transmembrane strands. As has been noticed with other membrane proteins (Cowan and Rosenbusch, 1994), there is a high concentration of aromatic residues at one end of the strands where they would begin to interact with the phospholipid headgroups (Fig. 5). The long loop regions tend to be located in the body of the protein away from the N and C termini. There are loose loops at both ends of the seventh strand which, based on electrophysiological observations, seems to slide up and down normal to the membrane during the voltage gating process (Zizi *et al.*, 1995). These loose loops allow for the motion and the proposed location of this strand closer to the N terminus. The turns between the first and the second and the ninth and the tenth strands are very tight with only one or three residues.

The Folding Patterns Are Consistent with Available Data

The folding pattern of yeast VDAC fits well with the electrophysiological data collected on yeast VDAC (Blachly-Dyson *et al.*, 1990; Peng *et al.*, 1992) (Fig. 4A). Those residues for which mutations changed the

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            1                                                                    50
wheat  MGGPGLYSGI GKKAKDLLYR DY..QTDHKF TLTTYTANGP AITATSTKKA
yeast  .MSPPVYSDI SRNINDLLNK DFYHATPAAF DVQTTTANGI ........KF
            51                                                                  100
wheat  DLTVGEIQSQ IKNKNITVDV KANSASNVIT TITADDLAAP GLKTILSFAV
yeast  SLKAKQPVKD .GPLSTNVEA KLNDKQTGL. GLTQGWSNTN NLQTKLEFAN
            101                                                                 150
wheat  PDQKSGKVEL QY.LHDYAGI NASIGLT.AN PVVNLSGAFG TSALA.....
yeast  LTP.GLKNEL ITSLTPGVAK SAVI.NTTFTE PFFTARGAFD LCLKSPTFVG
            151                                                                 200
wheat  .......... VGADVSLDTA TKNFAKYNAA LSYTNQDLIA SLNLNNKGDS
yeast  DLTMAHEGIV GGAEFGYDIS AGSISRYAMA LSYFAKDYSL GATLNNEQIT
            201                                                                 250
wheat  LTASYYHIVE KSGTAVGAEL THSFSSNENS LTFGTQHTLD PLTLVKARIN
yeast  TVDFFQNVNA FLQVGAKATM NCKLPNSNVN IEFATRYLPD ASSQVKAKVS
            251                                                     285
wheat  NSGKASALIQ HEFMPKSLCT ISAEVDT.KA IEKSSKVGIA IALKP
yeast  DSGIVTLAYK QLLRPGVTLG VGSSFDALKL SEPVHKLGWS LSFDA
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Fig. 3. An alignment of yeast and wheat VDAC sequences, showing that a good alignment can be achieved by introducing one segment deletion and one segment insertion into the primary sequence of wheat VDAC. The residues in bold letters are identical in both sequences.

open-state selectivity are inside the channel, while those residues whose mutations did not change the selectivity are in the outside of the channel. The folding pattern of human 1 VDAC (Fig. 4B) fits with the electrophysiological observations not yet published (Byrd, Blachly-Dyson, Colombini, and Forte, unpublished observations) in that substitutions of glutamate for lysine at either position 112 or 114 did not change the selectivity, while the same substitution at 118, a site very close to opening of the channel in the proposed folding pattern, changed the selectivity. This folding pattern is also consistent with the cleavage sites of trypsin, (between 108–119) and chymotrypsin (Y117 or Y172) on VDAC in intact mitochondria as reported by De Pinto *et al.* (1991). As far as their reported cleavage site of V8 protease is concerned, the proposed folding pattern agrees with their data but not their interpretation of the data. They found that a 22K peptide was detected by antibodies against VDAC after V8 protease treatment of intact mitochondria. Thus, the V8 protease cleavage site is in the same side of the membrane as the trypsin and chymotrypsin cleavage sites. In an effort to identify whether the V8 protease was acting on aspartate or glutamate residues, these

authors performed the cleavage under different conditions following the methods of Houmared *et al.* (1972). They found that the same peptide was obtained in Tris-HCl, pH 8, or in ammonium bicarbonate at pH 8.2, but not in ammonium acetate at pH4.0. These authors referred to Houmard *et al.* (1972) as "proteinase V8 specifically cleaves the peptide bond at carboxyl side of Glu at pH 4.0 and at the carboxyl side of both Glu and Asp at pH 8.0" and proposed the cleavage site to be D227 or D229. However, Houmard *et al.* reported that V8 protease "hydrolyzes only glutamoyl bonds in either ammonium bicarbonate (pH7.8) or ammonium acetate (pH 4.0)." Since cleavage was observed in ammonium bicarbonate at pH 8.2, the cleavage site could not be at an aspartate but must have been at a glutamate site. Their results are consistent with a V8 protease cleavage site at E176. This would result in a 22K polypeptide. In our proposed folding pattern, E176 is located at the opening of the pore on the same side of the membrane as the cleavage sites of trypsin and chymotrypsin.

Experiments on VDAC from very different sources have shown a very similar ion selectivity (Colombini, 1989). Current understanding of selectiv-

Fig. 4. The proposed folding patterns for VDAC from yeast (A), human (B), Neurospora (C), Dictyostelium (D), and maize (E), all of which are composed of an α-helix at the N-terminus and 12 β-strands. The space between dotted lines represents the mitochondrial outer membrane. Also shown are some experimental results which have been collected so far. Residues for which mutations altered the open state selectivity are boxed; residues for which mutations left the open state selectivity unchanged are circled. The predicted transmembrane strands of the 16-strand models of Rauch et al. (1994) are indicated in the folding patterns of human 1, yeast, Neurospora, and Dictyostelium VDAC by being boxed by dotted lines.

A yeast
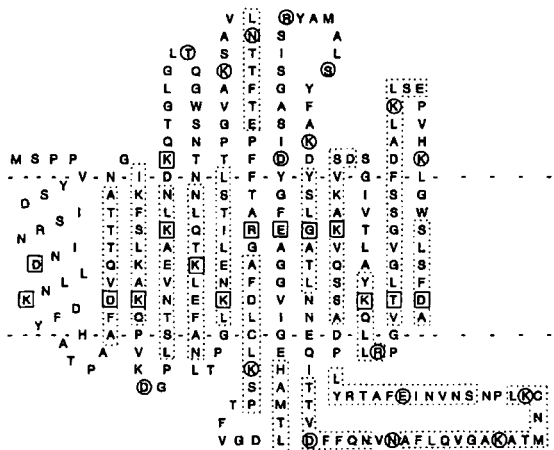
B Human 1
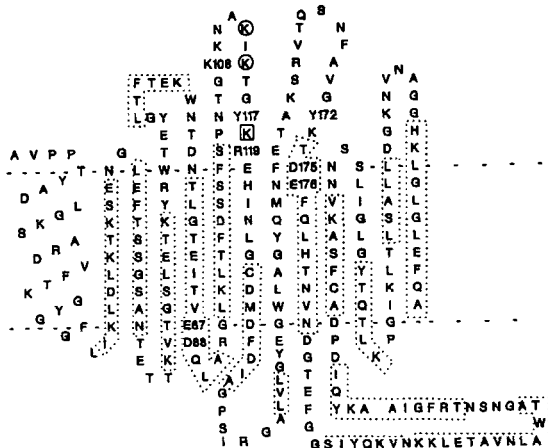
C Neurospora
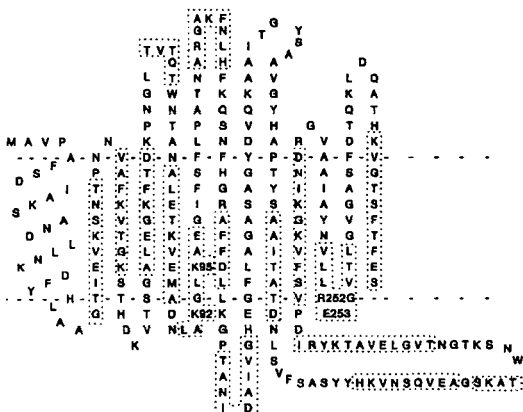
D Dictyostelium
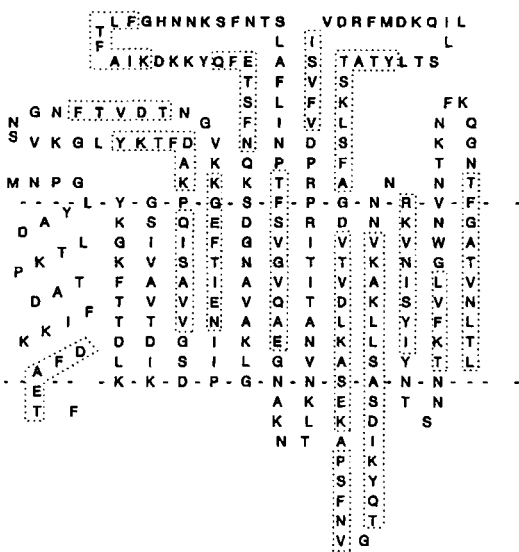
E Maize
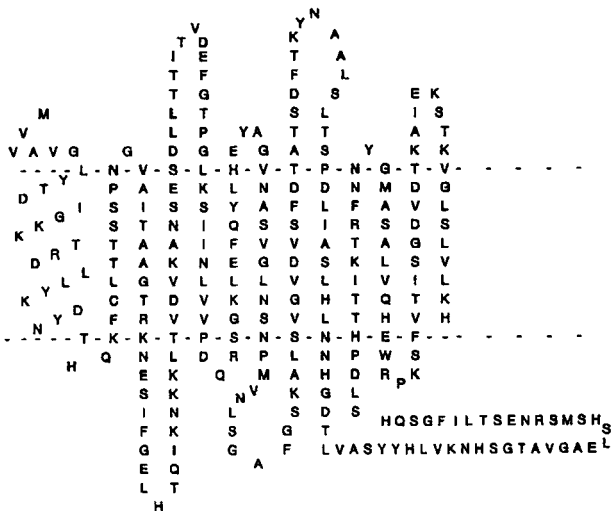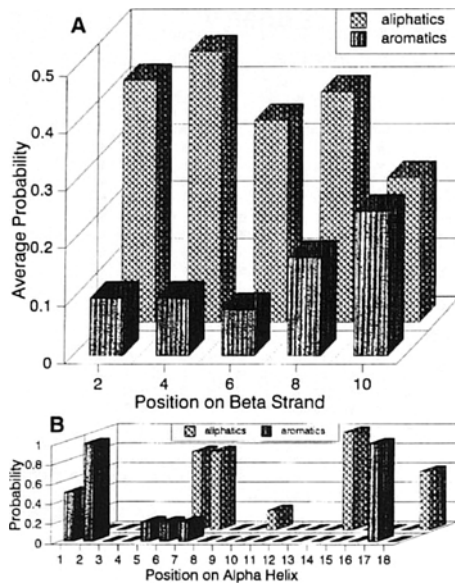
Fig. 5. The average distribution of aromatic (Phe, Tyr, His, and Trp) and aliphatic (Ile, Leu, Val, and Met) residues on the 12 proposed β-strands (A) and the N-terminal α-helix (B) in the folding patterns of VDAC from human 1, Neurospora, yeast, Dictyostelium, wheat, maize, and pea. There are 10 amino acids in each β-strand and 18 amino acids in each α-helix. The average probability of finding aromatic or aliphatic residues at a certain position was plotted against the number of the lipid-tail-facing rows (odd rows face the pore) on the β-strand in panel A and of every position on the α-helix in panel B. The probability for the β strands for each VDAC sequence was calculated by adding up the number of times a particular type of residue is present at a particular location on each β strand (starting from the same surface of the membrane) and dividing by 12, the number of strands. In order to achieve an appropriate weighting, the mean of the plant sequences was averaged with the values of the other four sequences.

Table I. Counted Total Net Charge Inside the Channel

Source	12-Strand model	16-Strand model[a]
Human 1	0	−1.0 (−2.5)[b]
Yeast	+1	+4.5
Neurospora	+2.5	+4.5
Dictyostelium	+1.5	+1.5
Pea	+2.5	
Wheat	+2.5	
Maize	+	
Potato	+2	

[a] Models of Rauch and Moran, 1994.
[b] Model of De Pinto et al., 1991.

## The Proposed Transmembrane Strands in the Primary Sequences from Different Species Show a Considerable Amount of Primary Sequence Homology

Figure 6 compares the primary sequences in each of the 13 proposed transmembrane strands from eight species. While there are many changes in the primary sequence, some residues are found in almost all sequences, most notably K19, G93L94K95 of yeast VDAC. In many places, the substitutions are conservative, such as hydrophobic residues for hydrophobic residues and hydrophilic residues for hydrophilic residues. The α helix is especially well conserved. The plant and the animal VDAC sequences have clearly evolved apart as reflected by the dual entries in the consensus sequences. The fungi, yeast and Neurospora, have similarities to both the animal and plant sequences. Dictyostelium is, by far, the most dissimilar.

DISCUSSION

VDAC is a monomer which uses a small amount of protein to form a large pore (Mannella, 1987; Thomas et al., 1991; Peng et al., 1992). So it is very likely that the pore consists of a single layer of protein. Mannella et al. (1989a,b) used electron microscopy of frozen-hydrated 2-dimensional crystals of VDAC from Neurospora to conclude that the channel is basically a cylinder with a 3.8 nm diameter from the center of one protein wall to the other. It seems to have a 0.5 nm thick shell of amino acid residues on either side. The inner diameter would thus be about 2.8 nm. This is consistent with both negative staining and functional

ity in large channels indicates that the selectivity is dominated by the net charge on the walls lining the aqueous pore (Zambrowicz and Colombini, 1993). Thus a simple estimation of this net charge by considering the dissociable groups facing the channel lumen should yield a net charge consistent with the observations. Table I shows that this net charge is consistent with a preference for anions for all but human VDAC. Since this calculation ignores known differences in the effect of charges on the wall on ion selectivity, it is a crude estimate. The fact that the calculated net charge is very close for VDAC from all sources indicates that the folding patterns are consistent with the observations on selectivity. Certainly, these calculations indicate that 16-strand models are not as consistent with the selectivity data as the folding patterns presented here.

A

$LY^A_S D^L_I GK^{S\,AK}_{K\,TR} DLL^N_Y KDY^N_Y$

worm	PS1I^ESXC^XFf^*G**
human 1	T*^*^**^^-*vfT*G*G
Neurospora	AF-*-a*^^N***^**F-
yeast	v*-*-SrNIN***^**F-
Dictyostelium	**^*^T*P-A*FiK**FA
wheat	**-G-**-^^***-r**Q
maize	**t*-**---***-***^
pea	**t*-**-^-***-***H

B

$K^L_P D^{LK}_{VT} TK^{SP}_{TA} N$ $V^{EFKS8}_{AITA} G^{S*E}_{K*S}$ $T^{GNLE}_{STDVE} ^{KA}K^{Y*D}_{F*S}$

worm	*1*S-*rAGD	*^^^^^Aa^H-	G^^^dv*^KI
human 1	^^*^^**^E*	1^^-^^*^A-	^*S^^T*^RW
Neurospora	Tie-^sN-^*	*-^^v-*-ST	-^A^^g*FT^
yeast	A-*-Q*T--*	1K^slKa-QP	--^-**L**
Dictyostelium	*^*T-F*GKY	gS-V-VTDI^	DsG-V*SIqP
wheat	*-T^-*Y--*	P-----St**	*V---*N-A-
maize	*-C^-*S^**	*----A*tr^	^V---*N-E-
pea	*-Tis*Y^^T	*----**t**	---i-vDt*-

$NKL^{QTE I}_{IIKL} EV^A_P$ $GLK^{V LDSSY}_{A FIYLH}$ $^{FT}_{IH} A^R_S VG^A_F D^L_A N$

worm	*Q**^V**N	***^T^^^-Y	P^^*T^*vW
human 1	*T*^^^^T*E	**1T-^^^^	E-iN1*C*MD
Neurospora	*A*E^-v*M^	***-*g-F^^	*-g*af-**L
yeast	*N*Q^--*f^	***N*^-t*L	*^*^ga-**C
Dictyostelium	GEfT-^NIi-	***-VaNGDS	*svG*Q*eg*
wheat	L*T-1SfA*-	SG***Q---	-N*-i*1T-*
maize	L*S--N-V*-	SG*1*-Q---	vN*-**1NS*
pea	V*a-1SfK*-	SG**^*Q---	-sS-**1K-*

C

$G^{FL}_{VG} AG^V_A S^{AGY}_{FDT}$ $P^T_D LIASL^I_N LN$ $D^{AL}_{ST} VKA^K_R VNN$

worm	DX^i**X^TX		
human 1	*W***YQMNF	DefQlHT-v*	**CfS******
Neurospora	*^^**-*^^^	**Ys*Ai^aT	V-F**g^i*D
yeast	*ivg*-E-^^	Ys*g*t*-Ne	**sSQv^aKV
Dictyostelium	N-N*TitiRP	G-vTvD*KaS	A-^1*******
wheat	a---*D^*1--	Q-*****-**	Lt****-i**
maize	S--vD*^---	*-*****H**	Ht-i*T-f**
pea	a^-*Di*---	D-**g**^**	Lt-**g-*T*

$GK^I_V s^L_A L^Y_V Q^O_H E$ $G^I_V TL^{GLGASF}_{SVEVDT}$ $^L_V GLSLA^F_L K^A_P$

worm			
human 1	SL^G^g^T^T	**K*t^S*LL	^**G*E^Q^
Neurospora	aA^AYN-LLR	*-**^-^^^^	-*T*fT^ES
yeast	*I-t^a^K*L	*-**-^g*^	^*W**S*D^
Dictyostelium	R*-NiS^IYN	NTKfV^*WNV	f*atvN-TL
wheat	**a*-*i*-*	1C*i-a----	-*iAi*-*-
maize	*Ma*-*--*-*	f-*i-gd---	-****v-*H
pea	**a*-*i*-*	1**i-S----	i*****-*-

Fig. 6. Comparisons of the primary sequences of the corresponding proposed transmembrane strands for various species. Only the proposed transmembrane segments are shown. The sequences begin from the N terminus with the α-helix (A) and continue with 12 β-strands (B, C). The top sequences represent consensus patterns for the corresponding strands. The symbol * stands for the residues which are identical to the one consensus residue. The symbols ^ and - stand for residues that are identical to the upper or lower of two possible consensus residues, respectively. Residues in lower case letters have very similar properties to those of the consensus residue(s). The rest of the residues are in upper case.

permeability measurements (Mannella *et al.*, 1992). Circular dichroism measurements indicate the presence of a high degree of β structure in VDAC (Mannella *et al.*, 1995). Alternating hydrophobic and hydrophilic patterns are required to form transmembrane β-strands lining the aqueous pore. About 10 amino acids are needed to form a transmembrane β-strand and about 20 amino acids are needed to form a transmembrane α-helix.

We found that VDAC from all species has very similar β-patterns with 12–15 peaks indicating locations of potential transmembrane β-strands in the primary sequences. This β-pattern is especially well conserved among species from the same kingdom of life. The similarity of the β-pattern for VDAC of species from different kingdoms is also very remarkable. Twelve regions of the primary sequence corresponding to the 12 transmembrane strands identified for yeast VDAC were identified in the VDAC sequence from each of the other species. These point to the possibility

that all VDAC molecules use the same basic β-strands to form the β-barrel pore. The N-terminal region from all the species has a very similar sequence that corresponds to the α helix proposed to form part of the wall of the aqueous pore (Blachly-Dyson *et al.*, 1990).

All the VDAC sequences from different organisms can be put into a very similar folding pattern to that previously proposed for yeast VDAC (Blachly-Dyson *et al.*, 1990: Peng *et al.*, 1992; Thomas *et al.*, 1993). This consists of an α-helix and 12 β strands forming a 13-strand barrel. These folding patterns not only obey expectations for a transmembrane water-filled channel such as no charged residues facing the phospholipid tails and no prolines in the secondary structure. They also have other desirable characteristics such as high probability of aromatic residues next to the surface and an appropriate charge in the lumen. Figure 5 shows the higher probability of finding aromatic residues at one end of the β strands and remarkably high probability (value of 1) of aromatic residues

at specific sites at both ends of the α helix. These 12 proposed transmembrane β-strands show substantial sequence similarity, even though the primary VDAC sequences from different kingdoms have very low homology to one another.

The proposed folding patterns are consistent with the available data. Both published and unpublished data on sites that affect selectivity identify sites that either face or do not face the stream of ions flowing through the channel. The folding patterns are consistent with all available results. More specifically, human 1 VDAC has a segment (108–118) that is quite different from the corresponding area in yeast VDAC. It has a good alternating pattern characteristic of a transmembrane strand (note extra peak in β pattern in Fig. 2A, denoted by arrow). If this did form a transmembrane strand, it would indicate a fundamentally different folding pattern for human 1 VDAC as compared to yeast VDAC. Specific tests by site-directed mutation showed that this strand is not transmembrane, indicating that the common folding pattern is likely (Fig. 4B). The proposed folding pattern for human 1 VDAC also fits with the protease cleavage sites identified by De Pinto et al. Therefore, the available experimental data also point to the validity for the concept of a common folding pattern for VDAC from different sources.

In contrast, competing 16 transmembrane β-strand models for VDAC (De Pinto et al., 1991; Rauch and Moran, 1994) are in conflict with the electrophysiological data collected on yeast VDAC in that the mutations of many amino acids in regions which they proposed to be inside the channel actually did not affect the open-state selectivity, and mutations of some other amino acids in regions which they proposed to be outside the channel, including some residues in the N-terminal α-helix, did affect the open-state selectivity. The proposed 16 strands in the Rauch and Moran models are indicated in dotted outlines in Fig. 4A–D. Note that some of the proposed strands include residues shown not to face the ion stream (circled residues) based on selectivity measurements (Blachly-Dyson et al., 1990; Peng et al., 1992). Some other residues shown to affect the selectivity (boxed residues) are not included in these 16 strands. There are other problems with these models. There are some adjacent charged amino acids deep inside the channel (E87D88, D175E176 of human 1 VDAC; R252E253 of Neurospora VDAC) and so by the nature of the β barrel one of each pair of charged residues must face the apolar portion of the membrane. For similar reasons, some other charged amino acids in the proposed transmem-

brane strands must face the phospholipid tails (D264 or K267 of yeast VDAC; K92 or K95 of Neurospora VDAC). These unfavorable interactions cast more doubt on the validity of the 16-strand models. Finally, these models propose the existence of unreasonably short transmembrane strands, the shortest of which only has three amino acids with which to span the membrane (Fig. 4B). Such a short loop might exist if it extended into the lumen of the channel so that the biophysical constraints of the alternating polar–nonpolar pattern no longer applied. However, there is no indication that VDAC has a loop extending into the lumen of the channel in analogy to the bacterial porin structure solved by X-ray crystallography (Cowan and Rosenbusch, 1994). An argument made in support of the 16-strand models is the assertion that VDAC has a very similar structure to that of bacterial porins. But, according to our analysis, VDAC has a very different β-pattern from that of the bacterial porins (Mannella et al., 1992). In addition, there is no significant sequence homology between VDAC and the bacterial porins (Forte et al., 1987) and the electrophysiological properties of these two channels are also very different (Mannella et al., 1992). Here we are trying to make a case for a similar folding pattern for a set of channel formers with almost identical properties but isolated from different species. To extrapolate to molecules with very different functional properties does not seem justified.

In summary, there are many indications that the 13 strand folding pattern proposed for yeast VDAC is conserved for VDAC from other species examined to date.

ACKNOWLEDGMENT

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