

MINI-REVIEW

Molecular Genetics of the VDAC Ion Channel: Structural Model and Sequence Analysis

Michael Forte,^{1,4} H. Robert Guy,² and Carmen A. Mannella³

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Abstract

The voltage-dependent anion-selective channel of the outer mitochondrial membrane provides a unique system in which to study the molecular basis of voltage gating of ion flow. We have cloned and sequenced a cDNA coding for this protein in yeast. From the derived amino acid sequence, we have generated a preliminary model for the secondary structure of the protein which suggests that the protein forms a " β -barrel" type structure. Comparison of the VDAC amino acid sequence with that of the bacterial porins has indicated that the two classes of molecules appear to be unrelated.

Key Words: Yeast VDAC; gene cloning; secondary structure; bacterial porins; sequence homology.

Introduction

The term "voltage-sensitive ion channel" refers collectively to a group of rather diverse proteins which form aqueous pathways for the movement of ions and other small molecules across biological membranes. The feature which distinguishes these channels from other types of membrane channels is that the pathway opens or closes in response to a change in the potential difference across the membrane. Although the function of these molecules has been the

¹Vollum Institute for Advanced Biomedical Research, The Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Road, Portland, Oregon 97201.

²Laboratory of Mathematical Biology, National Institutes of Health, Bethesda, Maryland 20205.

³Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201.

⁴To whom correspondence should be addressed.

subject of intense physiological study for over 30 years, our understanding of the molecular basis of voltage-sensitive ion channel function remains an abstraction necessitated by functional studies. How exactly is the voltage stimulus sensed and how does this cause the channels to open or close?

As is often the case when biochemical solutions are sought for complex biological phenomena, analysis of "simple" voltage-sensitive ion channels may provide us with initial answers to these questions. In this light, one particularly attractive system in which to study the basic molecular characteristics of voltage gating is provided by the voltage-dependent anion-selective channel (VDAC) present in the outer mitochondrial membrane of all eukaryotes. VDAC was first identified as the permeability pathway for small molecules between the cytoplasm and the intermembrane space (Schein *et al.*, 1976; Colombini, 1979). VDAC has subsequently been purified to homogeneity from the outer mitochondrial membrane of *Neurospora* (Freitag *et al.*, 1982) and rat liver (Linden *et al.*, 1982; Colombini, 1983; Roos *et al.*, 1982). VDAC is in most cases a major protein of the outer mitochondrial membrane with a molecular weight of approximately 30,000. The purified protein spontaneously inserts into artificial bilayers from detergent-containing solutions to produce channels identical to those assayed from outer mitochondrial membranes (for review see Benz, 1985).

VDAC is synthesized and inserted into the outer mitochondrial membrane as apparently are all components of this membrane, on free, cytoplasmic polysomes (Freitag *et al.*, 1982). Posttranslational insertion is not accompanied by processing of an NH₂ terminal signal sequence. In addition, insertion is not dependent on ATP or an electrical potential across the inner membrane (Freitag *et al.*, 1982; Mihara *et al.*, 1982; Gasser and Schatz, 1983).

Our aim has been to take advantage of the uniqueness of VDAC as an experimental system in which to study the voltage gating of ion channels and the elegance of molecular genetic analysis as is possible in the yeast *S. cerevisiae*. Using this combined approach, we hope to identify specific domains within the protein which are responsible for voltage gating, the location of these domains within the structure of the protein, and conformational changes that take place within the protein to cause it to open or close in response to a transmembrane voltage.

Yeast VDAC

Our initial approach was to develop procedures for the large-scale purification of VDAC from yeast outer mitochondrial membranes. The material for these preparations are commercial blocks of baker's yeast,

providing an almost unlimited supply of starting material. The procedures used are essentially modifications of the methods described by Freitag *et al.* (1983). In its pure form, VDAC from yeast has an apparent molecular weight of 29,000 as determined by SDS polyacrylamide gel electrophoresis, a size consistent with that previously estimated by Mihara *et al.* (1982) for yeast VDAC in partially purified preparations. The functional properties of purified yeast VDAC have been examined after insertion into a planar lipid bilayer. The single-channel conductance (4.5 nS), ion selectivity, and voltage sensitivity of yeast VDAC are essentially that observed for VDAC purified from other sources (Benz, 1985). A complete description of the biochemical and physiological characteristics of purified yeast VDAC will be presented elsewhere (Forte *et al.*, 1987).

Purified yeast VDAC has also been used to elicit a population of rabbit polyclonal antibodies. Serum from these immunized rabbits was then used to prepare affinity-purified antibodies which recognize yeast VDAC with high specificity. These affinity-purified antibodies have allowed us to isolate the gene coding for yeast VDAC by initially screening a yeast genomic library cloned in the expression vector λ gt11 (Forte and Guy, 1986). Sequences expressing VDAC epitopes fused to bacterial β -galactosidase were then used to isolate VDAC clones from both yeast cDNA and genomic libraries. VDAC clones were authenticated by their ability to hybrid-select mRNAs which, when translated *in vitro*, produce a protein which is immunoprecipitated by affinity-purified VDAC antibodies. VDAC clones were then sequenced by standard dideoxy techniques.

Figure 1 shows the nucleotide and amino acid sequence of yeast VDAC determined from a cDNA clone. The sequence contains one open reading frame of 883 nucleotides which produces a protein of 283 amino acids and has a molecular weight of 29,883, in good agreement with the size determined for the purified protein. In addition, our sequence matches precisely the sequence for yeast VDAC found by Mihara and Sato (1985) who have isolated the VDAC gene in their study of how proteins are localized in the outer mitochondrial membrane. Two aspects of the sequence deserve comment. First, as pointed out by Mihara and Sato (1985), the NH₂ terminal portion of the protein shows some homology to the NH₂ terminus of other outer mitochondrial membrane proteins. Work by Schatz and colleagues (for example, Roise *et al.*, 1986) has shown that the NH₂ terminal region of the presequence of imported mitochondrial proteins targets proteins to the mitochondria. NH₂ terminal regions of these proteins appear to adopt a unique structural conformation which is required for this targeting (Roise *et al.*, 1986; Von Heijne, 1986). Since outer membrane proteins are inserted without processing of a presequence, targeting of these proteins may be determined by the NH₂ terminal region of the mature protein which may also

Met	Ser	Pro	Pro	Val	Tyr	Ser	Asp	Ile	Ser	10
5'ATG	TCT	CCT	CCA	GTT	TAC	AGC	GAT	ATC	TCC	30
Arg	Asn	Ile	Asn	Asp	Leu	Leu	Asn	Lys	Asp	20
AGA	AAT	ATC	AAT	GAC	CTA	TTG	AAC	AAG	GAT	60
Phe	Tyr	His	Ala	Thr	Pro	Ala	Ala	Phe	Asp	30
TTC	TAT	GAT	GCT	ACC	CCA	GCT	GCC	TTT	GAT	90
Val	Gln	Thr	Thr	Thr	Ala	Asn	Gly	Ile	Lys	40
GTC	CAA	ACA	ACA	ACC	GCC	AAT	GCC	ATT	AAG	120
Phe	Ser	Leu	Lys	Ala	Lys	Gln	Pro	Val	Lys	50
TTC	TCA	TTG	AAG	GCT	AAA	CAG	CCT	GTC	AAA	150
Asp	Gly	Pro	Leu	Ser	Thr	Asn	Val	Glu	Ala	60
GAC	GGT	GCA	CTG	TCT	ACT	AAC	GTG	GAA	GCA	180
Lys	Leu	Asn	Asp	Lys	Gln	Thr	Gly	Leu	Gly	70
AAG	TTG	AAT	GAC	AAG	CAA	ACC	GCC	TTG	GCT	210
Leu	Thr	Gln	Gly	Trp	Ser	Asn	Thr	Asn	Asn	80
CTA	ACT	CAA	GCC	TGG	TCT	AAC	ACA	AAC	AAC	240
Leu	Gln	Thr	Lys	Leu	Glu	Phe	Ala	Asn	Leu	90
TTG	CAA	ACC	AAA	TTA	GAG	TTT	GCC	AAC	TTG	270
Thr	Pro	Gly	Leu	Lys	Asn	Glu	Leu	Ile	Thr	100
ACC	CCT	GCT	CTA	AAG	AAC	GAA	TTG	ATC	ACT	300
Ser	Leu	Thr	Pro	Gly	Val	Ala	Lys	Ser	Ala	110
TCT	TTG	ACT	GCA	GCC	GTC	GCC	AAG	TCC	GCC	330
Val	Leu	Asn	Thr	Thr	Phe	Thr	Gln	Pro	Phe	120
GTC	TTA	AAC	ACT	ACG	TTC	ACA	CAA	CCT	TTC	360
Phe	Thr	Ala	Arg	Gly	Ala	Phe	Asp	Leu	Cys	130
ITC	ACC	GCA	AGA	GGT	GCC	TTT	GAC	TTG	TGT	390
Leu	Lys	Ser	Pro	Thr	Phe	Val	Gly	Asp	Leu	140
TTG	AAG	TCA	GCA	ACA	TTT	GTT	GGT	GAC	TTA	420
Thr	Met	Ala	His	Glu	Gly	Ile	Val	Gly	Gly	150
ACT	ATG	GCC	CAC	GAA	GGT	ATT	GTT	GGT	GCC	450
Ala	Glu	Phe	Gly	Tyr	Asp	Ile	Ser	Ala	Gly	160
GCA	GAG	TTT	GGT	TAC	GAT	ATC	ACC	GCC	GCT	480
Ser	Ile	Ser	Arg	Tyr	Ala	Met	Ala	Leu	Ser	170
TCC	ATT	TCT	CGT	TAT	GCG	ATG	GCT	TTA	AGT	510
Tyr	Phe	Ala	Lys	Asp	Tyr	Ser	Leu	Gly	Ala	180
TAT	TTT	GCC	AAA	GAC	TAC	TGC	TTG	GGC	GCT	540
Thr	Leu	Asn	Asn	Glu	Gln	Ile	Thr	Thr	Val	190
ACA	TTG	AAC	AAC	GAG	CAA	ATA	ACT	ACC	GTT	570
Asp	Phe	Phe	Gln	Asn	Val	Asn	Ala	Phe	Leu	200
GAC	TTT	TTC	CAA	AAC	GTC	AAC	GCC	TTT	TTA	600
Gln	Val	Gly	Ala	Lys	Ala	Thr	Met	Asn	Cys	210
CAG	GTC	GCT	AAG	GCT	ACA	ATG	ATG	AAC	TGC	630
Lys	Leu	Pro	Asn	Ser	Asn	Val	Asn	Ile	Glu	220
AAA	CTA	CCT	AAC	TCC	AAT	GTC	AAC	ATC	GAA	660
Phe	Ala	Thr	Arg	Tyr	Leu	Pro	Asp	Ala	Ser	230
TTC	GCC	ACT	AGA	TAT	TTG	CCT	GAT	GCA	TCT	690
Ser	Gln	Val	Lys	Ala	Lys	Val	Ser	Asp	Ser	240
TCC	CAA	GTT	AAG	GCT	AAG	GTG	TCC	GAT	TCC	720
Gly	Ile	Val	Thr	Leu	Ala	Try	Lys	Gln	Leu	250
GGT	ATT	GTC	ACT	TTG	GCT	TAC	AAG	CAA	TTG	750
Leu	Arg	Pro	Gly	Val	Thr	Leu	Gly	Val	Gly	260
TTA	AGA	GCT	GCC	GTC	ACT	CTG	GCT	GTC	GCT	780
Ser	Ser	Phe	Asp	Ala	Leu	Lys	Leu	Ser	Glu	270
TCC	TCT	TTC	GAT	GCT	TTC	AAG	TTG	TCT	GAA	810
Pro	Val	His	Lys	Leu	Gly	Trp	Ser	Leu	Ser	280
CCT	GTT	CAC	AAG	CTA	GCT	TGG	TCT	TTG	TCC	840
Phe	Asp	Ala	***							
TTC	CAC	GCT	TGA	3'						

Fig. 1. The complete nucleotide and derived amino acid sequence of the yeast VDAC protein as determined by nucleotide sequencing of a cDNA coding for this protein.

adopt this unique conformation. Second, VDAC contains a large fraction of polar amino acids and lacks long stretches of hydrophobic residues which might be expected for membrane-spanning regions. This is in contrast to the observation that, by a number of criteria, the protein appears to be extremely hydrophobic (Forte, unpublished observations; Mihara *et al.*, 1982; Freitag *et al.*, 1982; Gasser and Schatz, 1983).

Structural Model of the VDAC Ion Channel

From the primary amino acid sequence shown in Fig. 1, preliminary models of the secondary structure of the VDAC molecule have been derived (Forte and Guy, 1986) employing a computer algorithm used to generate models of the colicin channel (Guy, 1983), the nicotinic acetylcholine receptor (Guy, 1984) and the voltage-sensitive Na channel (Guy and Seetharamulu, 1986). This method predicts which portions of the protein are α helices or β structures and which are exposed to water, buried inside protein, or exposed to lipid (Guy, 1985). There appear to be no segments of the protein which could form a hydrophobic or amphipathic α helix of sufficient length to span the membrane. There are, however, many segments which have alternating hydrophilic and hydrophobic residues and are long enough to span the membrane as β strands. By postulating 15–19 transmembrane β segments, a single VDAC protein can be folded into a “ β -barrel” type structure which has pore dimensions consistent with those determined by electron microscopy (Mannella *et al.*, 1984) and biophysical analysis (Colombini, 1980). In such a structure, as shown in Fig. 2, hydrophilic amino acids either line the water-filled pore of the channel or are in turn regions, while adjacent hydrophobic residues interact with the lipid bilayer. When adjusted to favor the β structure, the Delphi program (Garnier *et al.*, 1978) predicts that most of the putative transmembrane segments will be β strands and that the putative connecting segments will have coil and β turn conformations. Note that in the model shown in Fig. 2, the prolines are exclusively in turn regions. The strands have also been tilted relative to the membrane to give the optimal twist for a β -barrel type structure. If Fig. 2 were rolled into a cylinder, it would form a 19-stranded “ β -barrel” structure.

Comparison of the VDAC Sequence with Those of Bacterial Porins

VDAC has been commonly referred to as the “mitochondrial porin” by analogy to the porins of the outer membrane of gram-negative bacterial.

which also form passive diffusion channels. In addition, it is often speculated that VDAC may be evolutionarily related to the bacterial porins (Benz, 1985), implying a functional and structural relationship between these two classes of molecules. In fact, there are numerous differences between these channels. Functionally, in addition to variations in size and charge selectivity, VDAC channels are much more voltage dependent than the bacterial porin channels (Benz, 1985). Structurally, the lattice geometries of VDAC and bacterial porins in planar crystalline arrays are very different (Stevens *et al.*, 1977; Mannella *et al.*, 1983). In addition, bacterial porins form a complex with three channel openings on the extracellular surface which appears to fuse into one opening on the periplasmic surface (Engel *et al.*, 1985). The repeating unit usually observed for VDAC contains three channels which do not merge into a single channel at either membrane surface (Mannella *et al.*, 1983; Mannella *et al.*, 1984).

The primary amino acid sequence of yeast VDAC has been compared to those of the bacterial porins (Mannella and Auger, 1986) available in the National Biomedical Research Foundation (NBRF) protein database (Protein Identification Resource, Washington, D.C.) using the FASTP algorithm of Lipman and Pearson (1985). The FASTP algorithm finds the best alignments (five maxima) between two sequences by a diagonal matrix method; scores these alignments using Dayhoffs' log odds matrix for 250 accepted point mutations (highest score = Initial Similarity Score) (Dayoff, 1978); realigns the top-ranking segments, allowing for a minimal number of insertions and deletions; and rescores by using the point accepted mutation matrix (highest score = Optimal Similarity Score).

Table I shows the similarity scores obtained when the yeast VDAC sequences are compared with those of five bacterial porins by this algorithm along with the most related segments in each pair of sequences. On the basis of similarity scores, none of the VDAC/porin comparisons show the degree of relatedness as, for example, that shown by the different ADP/ATP carriers or the bovine ADP/ATP carrier and the uncoupling protein ("thermogenin") from rodent adipose tissue. These latter two sequences have recently been shown to be related (Aquila *et al.*, 1985). In these related sequences, the Initial Similarity Scores are greater than 90 and increase significantly after optimal alignment; the related regions in each case span a significant fraction of the molecules being compared. Initial Similarity Scores between VDAC and the bacterial porins all fall in the range of 29–37 and improve little or not at all with alignment optimization. Thus, there is nothing in this analysis to suggest that VDAC and the bacterial porins are related. Continued use of the term "mitochondrial porin" in reference to VDAC simply serves to confuse the properties of what appear to be two functionally, structurally, and evolutionarily distinct molecules.

Table I. Summary of Mitochondrial and Bacterial Protein Sequence Comparisons by FASTP^a

Query sequence ^b	Library sequence ^b	Similarity scores		Regions of similarity ^c	
		Initial	Optimal	Query sequence	Library sequence
Yeast VDAC	<i>E. coli</i> OmpA (MMECA)	35	35	274-278	33-37
	<i>E. coli</i> OmpF (MMECF)	29	30	82-90	102-110
	<i>E. coli</i> PhoE (MMECFE)	36	36	76-90	88-102
	<i>Salmonella</i> OmpA (MMEBAT)	35	35	274-278	33-37
	<i>Shigella</i> OmpA (MMEBAD)	37	44	75-86	155-166
Bovine ADP/ATP Carrier	ADP/ATP carrier	187	726	6-297	12-303
	<i>Neurospora</i> (XWNC)	198	737	7-294	13-300
	Yeast	92	208	118-294	18-198

^aFASTP software package was obtained from the Molecular Biology Computer Research Resource, Harvard School of Public Health, Dana-Farber Cancer Institute.

^bSequences were obtained from the NBRF library (identifiers in parentheses), except for yeast VDAC (Mihara and Sato, 1985; Forte and Guy, 1986), uncoupling protein (Aquila *et al.*, 1985), and yeast ADP/ATP carrier (Adrian *et al.*, 1986).

^cPositions of residues from the N-terminus.

Future Directions

Molecular genetic analysis of the yeast VDAC channel will proceed along three lines in the near future. First, nucleic acid probes for yeast VDAC will be used to isolate the VDAC gene from a number of other organisms. A number of such genes have already been isolated (Forte and Shafron, unpublished observations). Since VDAC proteins from a wide variety of organisms have almost identical biophysical properties, comparisons of the amino acid sequences of a number of VDAC proteins should point out conserved domains within the proteins which should be responsible for these conserved channel properties. In addition, such sequence comparisons will help modify the preliminary structure of VDAC shown in Fig. 2. Second, using the tricks of yeast molecular genetics (for example, Rothstein, 1983), it should be possible to construct yeast strains in which the gene encoding VDAC has been deleted. Finally, such deleted strains will serve as recipients for VDAC genes which have been mutated *in vitro* to localize domains within the protein which are responsible for the voltage-gating of ion flow.

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