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MINI-REVIEW

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

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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 *c*DNA 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.

Kew 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

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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 NH_2 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 polyconal 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 $\lambda gt11$ (Forte and Guy, 1986). Sequences expressing VDAC epitopes fused to bacterial β-galactosidase were then used to isolate VDAC clones from both yeast *c*DNA 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 *c*DNA 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 NH_2 terminal portion of the protein shows some homology to the NH_2 terminus of other outer mitochondrial membrane proteins. Work by Schatz and colleagues (for example, Roise *et al.*, 1986) has shown that the NH_2 terminal region of the presequence of imported mitochondrial proteins targets proteins to the mitochondria. NH_2 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 NH_2 terminal region of the mature protein which may also

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  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  CAT  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  GGC  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  CCA  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  GGC  TTG  GGT  210

  Leu  Thr  Gln  Gly  Trp  Ser  Asn  Thr  Asn  Asn   80
  CTA  ACT  CAA  GGC  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  GGT  CTA  AAG  AAC  GAA  TTG  ATC  ACT  300

  Ser  Leu  Thr  Pro  Gly  Val  Ala  Lys  Ser  Ala  110
  TCT  TTG  ACT  CCA  GGC  GTC  GCC  AAG  TCC  GCC  330

  Val  Leu  Asn  Thr  Thr  Phe  Thr  Gln  Pro  Phe  120
  GTC  TTA  AAC  ACT  ACG  TTC  ACA  GAA  CCT  TTC  360

  Phe  Thr  Ala  Arg  Gly  Ala  Phe  Asp  Leu  Cys  130
  TTC  ACC  GCA  AGA  GGT  GCC  TTT  GAC  TTG  TGT  390

  Leu  Lys  Ser  Pro  Thr  Phe  Val  Gly  Asp  Leu  140
  TTG  AAG  TCA  CCA  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  GGC  450

  Ala  Glu  Phe  Gly  Tyr  Asp  Ile  Ser  Ala  Gly  160
  GCA  GAG  TTT  GGT  TAC  GAT  ATC  AGC  GCC  GGT  480

  Ser  Ile  Ser  Arg  Tyr  Ala  Met  Ala  Leu  Ser  170
  TCC  ATT  TCT  CGT  TAT  GCC  ATG  GCT  TTA  AGT  510

  Tyr  Phe  Ala  Lys  Asp  Tyr  Ser  Leu  Gly  Ala  180
  TAT  TTC  GCC  AAA  GAC  TAC  TCC  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  TTC  TTC  CAA  AAC  GTC  AAC  GCC  TTT  TTA  600

  Gln  Val  Gly  Ala  Lys  Ala  Thr  Met  Asn  Cys  210
  CAG  GTC  GGT  AAG  GCT  AGA  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  CCT  GGC  GTC  ACT  CTG  GGT  GTC  GGT  780

  Ser  Ser  Phe  Asp  Ala  Leu  Lys  Leu  Ser  Glu  270
  TCC  TCT  TTC  GAT  GCT  TTG  AAG  TTG  TCT  GAA  810

  Pro  Val  His  Lys  Leu  Gly  Trp  Ser  Leu  Ser  280
  CCT  GTT  CAC  AAG  CTA  GGT  TGG  TCT  TTG  TCC  840

  Phe  Asp  Ala  ***
  TTC  GAC  GCT  TGA  3'
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Fig. 1. The complete nucleotide and derived amino acid sequence of the yeast VDAC protein as determined by nucleotide sequencing of a *c*DNA coding for this protein.

adopt this unique conformation. Second, VDAC contains a large fraction of polar amino acids and lacks long stretches of hydophobic 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.

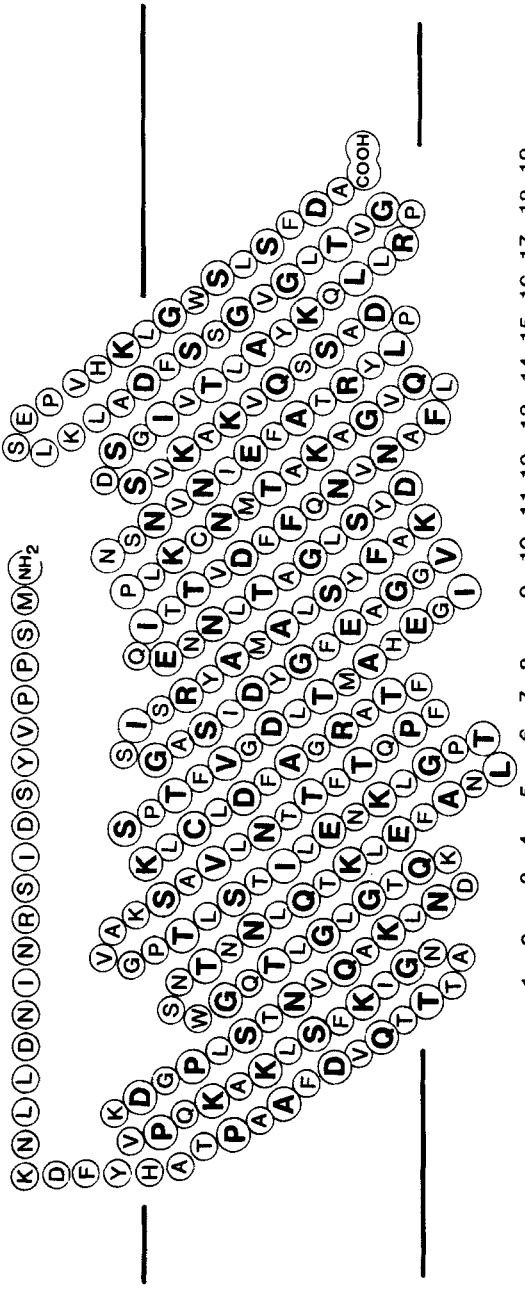

Fig. 2. Preliminary structural model of the secondary structure of the VDAC protein as determined by the Delphi computer algorithm (Guy, 1984). Large letters in larger circles indicate residues proposed to line the water-filled core of the channel. Small letters in smaller circles indicate residues proposed to interact primarily with the lipid bilayer.

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	*E. coli* OmpA (MMECA)	35	35	274–278	33–37
	E. coli OmpF (MMECF)	29	30	82–90	102–110
	E. coli PhoE (MMECPE)	36	36	76–90	88–102
	Salmonella OmpA (MMEBAT)	35	35	274–278	33–37
	Shigella OmpA (MMEBAD)	37	44	75–86	155–166
Bovine ADP/ATP Carrier	ADP/ATP carrier *Neuropsora* (XWNC)	187	726	6–297	12–303
	Yeast	198	737	7–294	13–300
	Uncoupling protein	92	208	118–294	18–198

[a] FASTP software package was obtained from the Molecular Biology Computer Research Resource, Harvard School of Public Health, Dana-Farber Cancer Institute.
[b] Sequences 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).
[c] Positions 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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