REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	2/19/99	final report 10/1/89 to 5/31/96

4. TITLE AND SUBTITLE
Probing the Voltage Gating and Modulation of a Voltage Dependent Channel

5. FUNDING NUMBERS
N00014-90-J-1024

6. AUTHOR(S)
Marco Colombini

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Dept. Biology, University of Maryland, College Park
MD 20742

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

VDAC channels form aqueous pathways through the mitochondrial outer membrane. These channels are formed by 30kDa protein monomers. The channels are selective for anions and will allow a variety of metabolites including ATP to cross the membrane. The channel structure is mostly formed by a beta barrel and consists of 1 alpha helix and 13 beta strands. Channel gating results in channels that have a lower overall conductance but inverted selectivity so that ATP and many anionic metabolites have drastically reduced permeability. There are 2 fundamental gating processes, one functioning at positive and the other at negative potentials. Voltage gating results from the movement of a large domain of net positive charge out from within the membrane to the membrane surface. A variety of agents, including NADH and intermembrane-space proteins, modulate the properties of VDAC, usually favoring channel closure. These agents are believed to be part of a complex regulatory system that controls mitochondrial function.

14. SUBJECT TERMS
VDAC, voltage-gating, mitochondrion, channel, structure

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
unclassified	unclassified	unclassified	unlimited

Final Report

Grant # N00014-90-J-1024

Principal Investigator: Marco Colombini

Institution: University of Maryland

Grant Title: Probing the Voltage Gating and Modulation of a Voltage Dependent Channel

Award Period: Oct. 1, 1989 to May 31, 1996

Objectives:

The overall objective was to learn about the structure, the function, and the regulation of the mitochondrial channel called VDAC. Specific goals were:
1. Identify the structure of the channel and the structural changes associated with voltage gating
2. Explore the nature of selectivity in large channels
3. Identify the proteins in the intermembrane space that modulate VDAC and the mitochondrial outer membrane permeability
4. Explore the ability of VDAC to regulate the flow of organic molecules that serve as metabolites

Approach:

The primary approach was to use the reconstitution of VDAC channels into solvent-free planar phospholipid membranes in order to analyze the properties of the channels in details. These properties are probed by site-directed mutations, chemical modification, and by the use of modulators. The properties of the channels in isolated mitochondria and whole cells were also examined.

Accomplishments:

By forming 2-dimensional crystals of VDAC in native membranes and then freeze-drying and shadowing them followed by examining platinum replicas, we were able to see the surface structure of both sides of the VDAC crystals. This gave us new insights into the channel's structure. It also allowed us to determine that a single protein monomer forms a channel. We confirmed the molecularity of the channel by using functional mutants and attempting to form hybrid channels.

By using site-directed mutations, we identified the regions of the channel that form the pore lining and those located outside the channel. We also demonstrated that large portions of the pore move upon voltage-gating. We used 3 independent approaches: mutation effects on selectivity, on voltage-gating, and on motion by using

cysteine mutagenesis followed by biotinylation and probing with streptavidin (the latter was not supported by Navy funds). All these agree very well. This structural information fits very well with the functional changes and the energetic requirements for voltage-gating to take place.

We discovered that NADH, but not the oxidized form NAD+,can modulate VDAC by favoring channel closure. This effect was shown not only with reconstituted channels but also on intact mitochondria. In order to make the measurements on intact mitochondria we had to develop the only methods to measure outer membrane permeability quantitatively. We also showed that the Mg-NADPH complex has similar effects.

We localized to the intermembrane space, a proteinaceous factor that modulated the properties of VDAC and favors VDAC closure. We partially purified the active component and learned a great deal about its properties. This information would later allow us to identify candidate proteins and prove that at least 2 have modulator activity. We now believe that there are a family of proteins with this activity. We also demonstrated that the partially-purified material reduced the permeability of the mitochondrial outer membrane and thus reduced ADP-dependent respiration.

We developed a theory for ion permeation through large channels. This theory requires only one fitting parameter and that must be within a narrow range, consistent with experimental results. With that, the theory could predict reversal potentials under a vide variety of conditions of salt, ion activity, activity gradient, anion valency and net charge on the channel wall. No other theory comes close to this degree of experimental agreement.

We discovered a novel catalytic property which we term, auto-directed insertion. VDAC channels can accelerate and directed the insertion of VDAC into phospholipid membranes. This acceleration can reach 10 orders of magnitude and thus may be important in ensuring correct intracellular targeting. The determination of the direction of insertion manifests itself in the uniform orientation of hundreds of channels in a membrane but that orientation varies from membrane to membrane because the initial insertion step is not directed.

We also studied how ionic flow through VDAC biased the gating process. The bias could be attributed to kinetic energy imparted to the walls of the channel, including the voltage sensor. This kinetic energy would favor closure by one or the other gating process depending on the direction of flow. These results are consistent with the gating mechanism worked out through many other experiments.

We demonstrated that VDAC gating results primarily in an inversion in ion selectivity and only secondarily in a reduction in pore size. It is this inversion in selectivity that is responsible for VDAC gating to be able to control the flux of the organic anions such as ATP, succinate, citrate, and phosphate. These findings point to a regulatory process at the outer membrane that can be used to augment or diminish mitochondrial

function in cells.

By using the ability of aluminum hydroxide to hold VDAC channels open, we were able to provide evidence that VDAC closure is an early event in the regulation of yeast mitochondria. When transferred from growth on glycerol to growth on glucose, the addition of aluminum hydroxide delayed this transition by an hour.

Conclusions:

We have gained a great deal of insight into the structure and molecular mode of action of VDAC channels. We have found extensive evidence that VDAC channels can regulate metabolite flux through the mitochondrial outer membrane and this may be used by cells to regulate overall mitochondrial energy production.

Significance:

The molecular basis for channel gating is an important biophysical problem. We have largely solved this problem for the VDAC channel. Regulation of mitochondrial function at the level of the outer membrane may be important for optimal cell function and for apoptosis. The insights that we have gained will allow us to further elucidate this novel regulatory process by which VDAC controls mitochondrial function.

Award Information:

1992- Departmental Outstanding Research Award to Mingyao Liu
1995- College of Life Sciences Faculty Award for Excellence in Research to Marco Colombini
1996- Departmental Outstanding Research Award to Anchin Lee

Publications:

a) full-length papers

Liu, M. and Colombini, M. 1991. Voltage gating of the mitochondrial outer membrane channel VDAC is regulated by a very conserved protein. American Journal of Physiology, 260 (Cell Physiology 29): C371-C374.

Thomas, L., Kocsis, E., Colombini, M., Erbe, E., Trus, B.L. and Steven, A.C. 1991. Surface topography and molecular stoichiometry of the mitochondrial channel, VDAC, in crystalline arrays. Journal of Structural Biology, 106: 161-171.

Wunder, U.R. and Colombini, M. 1991. Patch-clamping in liposomes containing whole mitochondrial membranes. Journal of Membrane Biology, 123: 83-91.

Peng, S., Blachly-Dyson, E., Colombini, M. and Forte, M. 1992. Determination of the number of polypeptide subunits in a functional VDAC channel from

Saccharomyces cerevisiae. Journal of Bioenergetics and Biomembranes, 24: 27-31.

Liu, M.Y. and Colombini, M. 1992. A soluble protein increases the voltage dependence of the mitochondrial channel, VDAC. Journal of Bioenergetics and Biomembranes, 24: 41-46.

Liu, M.Y. and Colombini, M. 1992. Regulation of mitochondrial respiration by controlling the permeability of the outer membrane through the mitochondrial channel, VDAC. Biochimica et Biophysica Acta, 1098: 255-260.

Mannella, C.A., Forte, M. and Colombini, M. 1992. Toward the molecular structure of the mitochondrial channel, VDAC. Journal of Bioenergetics and Biomembranes, 24: 7-19.

Peng, S., Blachly-Dyson, E., Colombini, M. and Forte, M. 1992. Large scale rearrangement of protein domains is associated with voltage gating of the VDAC channel. Biophysical Journal, 62: 123-153.

Bureau, M.H., Khrestchatisky, M., Heeren, M.A., Zambrowicz, E.B., Kim, H., Grisar, T.M., Colombini, M., Tobin, A.J. and Olsen, R.W. 1992. Isolation and cloning of a voltage-dependent anion channel-like M_r 36,000 polypeptide from mammalian brain. Journal of Biological Chemistry, 267: 8679-8684.

Blumenthal, A., Kahn, K., Beja, O., Galun, E. Colombini, M., and Breiman, A. 1993. Purification and characterization of the voltage-dependent anion-selective channel (VDAC) protein from wheat mitochondrial membranes. Plant Physiology, 101: 579-587.

Thomas, L., Blachly-Dyson, E., Colombini, M., and Forte, M. 1993. Mapping of residues forming the voltage sensor of the VDAC ion channel. Proceedings of the National Academy of Sciences U.S.A., 90: 5446-5449.

Holden, M.J., and Colombini, M. 1993. The outer mitochondrial membrane channel, VDAC, is modulated by a protein localized in the intermembrane space. Biochimica et Biophysica Acta, 1144:396-402.

Zambrowicz, E.B. and Colombini, M. 1993. Zero-current potentials in a large membrane channel: a simple theory accounts for complex behavior. Biophysical Journal, 65: 1093-1100.

McEnery, M.W., Dawson, T.M., Verma, A., Gurley, D., Colombini, M., and Snyder, S.H. 1993. Mitochondrial voltage-dependent anion channel: immunochemical and immunohistochemical characterization in rat brain. Journal of Biological Chemistry, 268: 23289-23296.

Zizi, M., Forte, M., Blachly-Dyson, E. and Colombini, M. 1994. NADH regulates

the gating of VDAC, the mitochondrial outer membrane channel. Journal of Biological Chemistry, 269:1614-1616.

Liu, M.Y., Torgrimson, A. and Colombini, M. 1994. Characterization and partial purification of the VDAC-channel-modulating protein from calf liver mitochondria. Biochimica et Biophysica Acta, 1185:203-212.

Lee, A., Zizi, M. and Colombini, M. 1994. β-NADH decreases the permeability of the mitochondrial outer membrane to ADP by a factor of 6. Journal of Biological Chemistry, 269: 30974-30980.

Zizi, M., Thomas, L., Blachly-Dyson, E., Forte, M. and Colombini, M. 1995. Oriented channel insertion reveals the motion of a transmembrane beta strand during voltage gating of VDAC. Journal of Membrane Biology, 144:121-129.

Krueger, S., Ankner, J.F., Satija, S.K., Majkrzak, C.F., Gurley, D., and Colombini, M. 1995. Spectral reflection of neutrons from a bilayer containing components from the outer mitochondrial membrane. Langmuir, 11:3218-3222.

Song, J. and Colombini, M. 1996. Indications of a common folding pattern for VDAC channels from all sources. Journal of Bioenergetics and Biomembranes, 28:153-161.

Ahmadzadeh, M., Horng, A., and Colombini, M. 1996. The Control of Mitochondrial Respiration in Yeast: A Possible Role of the Outer Mitochondrial Membrane. Cell Biochem. Funct., 14: 201-208.

Xu, X., and Colombini, M. 1996. Self-catalyzed insertion of proteins into phospholipid membranes. Journal of Biological Chemistry, 271: 23675-23682.

Lee, A., Xu, X., and Colombini, M. 1996. The role of pyridine dinucleotides in regulating the permeability of the mitochondrial outer membrane. Journal of Biological Chemistry, 271: 26724-26731.

Rostovtseva, T. and Colombini, M. 1996. ATP flux is controlled by a voltage-gated channel from the mitochondrial outer membrane. Journal of Biological Chemistry, 271: 28006-28008.

Lee, A., and Colombini, M. 1997. An unique method for determining the permeability of the mitochondrial outer membrane. Methods in Cell Science, 19: 71-81.

Ryerse, J., Colombini, M., Hagerty, T., Nagel, B., and Liu, T.T. 1997. Isolation and characterization of the mitochondrial channel, VDAC, from the insect *Heliothis virescens*. Biochimica et Biophysica Acta, 1327: 193-203.

Hodge, T. and Colombini, M. 1997. Regulation of metabolite flux through voltage-gating of VDAC channels. Journal of Membrane Biology, 157: 271-279.

Rostovtseva, T. and Colombini, M. 1997. VDAC channels mediate and gate the flow of ATP: implication on regulation of mitochondrial function. Biophysical Journal, 72: 1954-1962.

Zizi, M., Byrd, C., Boxus, R., and Colombini, M. 1998. The voltage-gating process of the VDAC channel is sensitive to ion flow. Biophysical Journal, 75: 704-713.

b) chapters in books

Colombini, M. 1991. Aluminum and membrane channels. In: Aluminum in Chemistry Biology and Medicine A series of advances Vol. 1 (Nicolini, M., Zatta, P.F. and Corain, B., eds.) pp. 33-43. Cortina International, Verona Italy and Raven Press, New York.

Colombini, M., Peng, S., Blachly-Dyson, E. and Forte, M. 1992. Probing the molecular structure and structural changes of a voltage-gated channel. In: Methods in Enzymology "Ion Channels" Vol. 207 chapt. 29 (Rudy, B. and Iverson, L.E., eds.) pp. 432-444. Academic Press Inc. Orlando, FL.

Colombini, M. 1994. The mitochondrial voltage-dependent anion-selective channel. In: Advances in Chemistry "Biomembrane Electrochemistry" Vol. 235 Chapt. 12 (Blank, M. and Vodyanoy, I. eds.) pp. 245-258. American Chemical Society, Washington D.C.

Colombini, M. 1994. Anion channels in the mitochondrial outer membrane. In: Current Topics in Membranes Vol. 42 chapt. 4 (Guggino, W.G. ed.) pp 73-101. Academic Press, Inc. San Diego, CA.

Colombini, M. 1994. Structure and function of the VDAC ion channel. In: NATO ASI Series "Molecular Biology of Mitochondrial Transport Systems" Vol. H83 (Forte, M. and Colombini, M. eds.) pp 281-296. Springer Verlag, Berlin Heidelberg.

Thomas, L., Blachly-Dyson, E., Colombini, M. and Forte, M. 1994. Probing for the voltage sensor in a mitochondrial channel, VDAC, using site-directed mutagenesis. In: NATO ASI Series "Molecular Biology of Mitochondrial Transport Systems" Vol. H83 (Forte, M. and Colombini, M. eds.) pp 229-245. Springer Verlag, Berlin Heidelberg.

Colombini, M., Blachly-Dyson, E. and Forte, M. 1996. VDAC, a channel in the outer mitochondrial membrane. In: "Ion Channels" Vol. 4 (Narahashi, T. ed.) pp 169-202. Plenum Publishing Corp., New York, NY.

c) abstracts:

Thomas, L., Blachly-Dyson, E., Colombini, M. and Forte, M. 1991. Probing for the voltage sensor of the VDAC ion channel by site-directed mutagenesis. Biophys. J., 59:215a.

Liu, M. and Colombini, M. 1991. The modulation of the mitochondrial outer membrane channel, VDAC, by a highly conserved protein from mitochondrial fractions. Biophys. J., 59: 93a.

Peng, S., Blachly-Dyson, E., Colombini, M. and Forte, M. 1991. Inferring the structural changes in VDAC, associated with the channel's gating process, through site-directed mutagenesis. Biophys. J., 59: 215a.

Colombini, M. 1991. Molecular insights into the structure and mode of action of the mitochondrial channel, VDAC. Biophys. J., 59: 117a.

Liu, M.Y. and Colombini, M. 1991. The permeability of the mitochondrial outer membrane to adenine nucleotides is regulated by a soluble mitochondrial protein. J. Cell Biol., 115: 298a.

Thomas, L., Blachly-Dyson, E., Colombini, M. and Forte, M. 1992. Identification of protein domains that form the voltage sensor of the mitochondrial channel, VDAC. Biophys. J., 61: A396.

Colombini, M., Blachly-Dyson, E., Peng, S., Thomas, L., and Forte, M. 1992. The structure and gating mechanism of the VDAC channel as revealed by site-directed mutations. Biophys. J., 61: A415.

Liu, M.Y., and Colombini, M. 1993. Isolation and purification of a VDAC channel-modulating protein from calf liver mitochondria. Biophys. J., 64: A78.

Zambrowicz, E.B., and Colombini, M. 1993. A novel description of ion flow through large channels. Biophys. J., 64: A327.

Lee, A.C., Zizi, M., and Colombini, M. 1993. NADH regulates the permeability of the mitochondrial outer membrane by controlling the gating of VDAC channels. Molec. Biol. Cell, 4: 104a.

Zizi, M., Thomas, L., Blachly-Dyson, E., Forte, M, and Colombini, M. 1993. Autodirected insertion of a mitochondrial membrane protein in phospholipid membranes. Molec. Biol. Cell, 4: 424a.

Zizi, M., Byrd, C. and Colombini, M. 1994. The voltage gating of VDAC is sensitive to ion flow. Biophys. J., 66: A247.

Zizi, M., Blachly-Dyson, E., Forte, M. and Colombini, M. 1994. Evidence for a functional sliding motion of a β-strand during voltage gating of the VDAC channel. Biophys. J., 66: A135.

Lee, A., Zizi, M. and Colombini, M. 1994. NADH controls mitochondrial respiration by regulating the outer membrane permeability to ADP. Biophys. J., 66: A21.

Colombini, M. and Forte, M. 1995. The structural change responsible for voltage gating in VDAC. Biophys. J., 68: A352.

Lee, A., Heda, M.H. and Colombini, M. 1995. The permeability of mitochondrial outer membrane is regulated by NADH, NADPH and oncotic pressure. Biophys. J., 68: A399.

Song, J.M. and Colombini, M. 1995. Indications of a common folding pattern for VDAC channels from all sources. Biophys. J., 68: A145.

Xu, X., and Colombini, M. 1996. The insertion of VDAC channels into phospholipid membranes is catalyzed by pre-inserted channels. J. Gen. Physiol. (in press)

Rostovtseva, T. and Colombini, M. 1996. Flux of ATP through VDAC channels in the open and closed state. Biophys. J., 70: A1.

Song, J., Forte, M., Blachly-Dyson, E. and Colombini, M. 1996. Probing the gating process of VDAC by using the biotin-streptavidin system. Biophys. J. 70: A2.

Xu, X. and Colombini, M. 1996. VDAC:a channel-forming protein with an auto-directed insertion mechanism. Biophys. J. 70: A257.