BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **243,** 258–263 (1998)
ARTICLE NO. RC977926

Novel Aspects of the Electrophysiology of Mitochondrial Porin

György Báthori,[1] Ildikó Szabó, Ibolya Schmehl,[2] Francesco Tombola, Vito De Pinto,* and Mario Zoratti[3]

*CNR Unit for Biomembranes, Department of Biomedical Sciences, University of Padova, Italy; and *Institute of Biochemical and Pharmaceutical Sciences, University of Catania, Italy*

Received November 10, 1997

The recent findings that mitochondrial porin, VDAC, participates in supramolecular complexes and is present in the plasmamembrane need to be reconciled with its biophysical properties. We report here that VDAC often displays previously unobserved or unappreciated behaviors. Reconstituted VDAC can: a) exhibit fast gating when in any of many conductance substates; b) close completely, although briefly, on its own; c) close for long periods, in the presence of König's polyanion; d) take several milliseconds to re-open when an applied transmembrane potential is switched off; e) be desensitized by prolonged exposure to high voltages, so that it will not re-open to the full conductance state upon subsequent return to zero voltage; f) display polarity-dependent voltage-induced closure. These behaviors are especially noticeable when the observations are conducted on a single reincorporated channel, suggesting that interactions between copies of VDAC may play a role in determining its electrophysiological properties. Any model of VDAC's structure, gating and function should take these observations into account.

Key Words: **mitochondrial porin; substates; gating kinetics; bilayer.**

INTRODUCTION

Mitochondrial porin (voltage-dependent anion channel, VDAC) has enjoyed renewed attention since the discovery that it partecipates in supramolecular complexes and that it is present in non-mitochondrial cellular membranes (1-3). VDAC is a component of the mitochondrial benzodiazepine receptor (4,5) and of complexes comprising the adenine nucleotide translocator and kinases (6). Either one of these supramolecular assemblies might coincide with the mitochondrial permeability transition pore/mitochondrial megachannel (5, 7-11), thought to be involved in apoptosis (12, 13). The presence of porin in the plasmamembrane of several cells, although still debated (14), is supported by biochemical (15) immunohistochemical (1) and electrophysiological (16, 17) data. The properties of isolated plasma membrane porin are identical to those of the mitochondrial counterpart (17; our unpublished results).

A large number of biophysical, structural and genetic studies (reviews:18-21) has dealt with this channel. The electrophysiology of VDAC has invariably been studied via isolation and reconstitution in artificial bilayers, mainly planar ones. In the vast majority of experiments many copies of the channel were allowed to insert into the membrane. The electrophysiological properties of VDAC, as currently perceived by the scientific public, may be outlined as follows: Monomeric (22,23) porin incorporates into artificial membranes in a self-catalytic process (24,25 and ref.s therein), forming water-filled channels with a maximal conductance of approximately 4.5 nS in 1 M KCl. The pore is voltage-dependent: it occupies the highest conductance, slightly anion-selective state at transmembrane potentials close to zero, and it is reversibly driven to lower-conductance states by voltages higher than about 20 mV. The voltage dependence is symmetrical, i.e., the polarity of the applied voltage is irrelevant, despite the fact that the porin inserts in an oriented manner (26). VDAC returns to the fully open state upon elimination of the transmembrane voltage, in a process considered to be almost instantaneous. Otherwise, VDAC is generally considered to be a "slow" channel (although no

[1] Present address: Department of Physiology, Semmelweis Medical University, Budapest, Hungary.

[2] Present address: Department of Atomic Physics, Eötvös Lorand University, Budapest, Hungary.

[3] To whom correspondence should be addressed: Department of Biomedical Sciences, University of Padova, via G. Colombo 3, 35121 Padova, Italy. Fax: + 39-49-8276049; E-mail: zoratti@civ.bio.unipd.it.

Abbreviations used: VDAC: voltage dependent anion channel (mitochondrial porin); KPA: König's polyanion; BLM: black lipid membrane.

0006-291X/98 $25.00

kinetic study has been published), with mean residence times in the time scale of several seconds or minutes. Multiple, mostly cation-selective substates exists (19), although some publications (e.g., 27-29) may convey the impression that only one substate is present. The channel never closes completely: while VDAC in substates is poorly permeable to ATP (28,29), all the possible conformations of the channel maintain the ability to conduct an easily measurable flow of small ions such as K^+ and Cl^- (e.g. 19). This last property in particular is difficult to reconcile with the presence of VDAC in the plasmamembrane and in the sarcoplasmic reticulum (2).

In the course of our work (approx. 600 experiments) with reconstituted VDAC we have often observed several characteristic behaviors at variance with the description outlined above, which we report here. Only few papers (10, 30, 31) have previously described some non-canonical features of VDAC's behavior, receiving little attention. Since the properties described in this paper are prominent and reproducible, any model of VDAC's function, structure and dynamics should take them into account.

MATERIALS AND METHODS

Beef heart and rat liver mitochondrial porin was purified as previously described (32,33) using Triton X100 or Octylglucoside as detergents. The porin preparations displayed a single band on silver-stained SDS gels (not shown). BLM experiments were performed using a standard electrophysiological planar bilayer apparatus (34). Bilayers of approx. 300 pF capacity were prepared by painting azolectin (purified Sigma azolectin type II-S) or phosphatidylethanolamine (from brain; Avanti Polar lipids) dissolved in decane or chloroform across a smoothed hole in a Teflon film separating two 3-ml chambers carved in a Teflon block. The Ag/AgCl electrodes were connected to the solution by agar bridges. A few micrograms of purified porin were added to one of the chambers (*cis* side) and after incorporation of one or a few channels the *cis* chamber was perfused with approx. 20 ml of medium in order to prevent further incorporation. Channel activity was monitored and recorded on tape for off-line analysis (Axon Pclamp 6.0). All the voltages reported here refer to the *cis* side, zero being assigned by convention to the *trans* (grounded) side. The standard experimental medium contained KCl (usually 0.1 or 1 M), 0.1 mM $CaCl_2$, 20 mM HEPES/K^+, pH 7.2. The ion selectivity measurements were made under asymmetrical ionic conditions (usually 390 *cis* vs. 100 *trans* mM KCl). The polyanion was a generous gift of Prof. Tamás König.

RESULTS

An important discrepancy between our observations and the canonical model of VDAC behavior summarized in the introduction is the frequent occurrence of fast gating. In the absence of a better criterion, we arbitrarily consider as "fast" all gating possessing a time constant or a mean residence time below 1 sec. for at least one of the states involved. Fast gating was often observed also in multi-channel experiments, in which however slow modes predominated, but it was the dominant behavior observed with single-channel incorporations, i.e., when the planar membrane harbored only one pore. The experiments from which the traces in Fig. 1 were taken were performed under these conditions. Fast gating was exhibited mainly between substates. The variety of substates and of kinetic characteristics displayed was enormous. Despite extensive analysis and kinetic characterization, no univocal relationship between certain kinetic parameters and a certain set of conductance levels could be established. Fig. 1 is not meant to present a complete survey of observations, but only to illustrate the most often encountered types of behavior, which might be arbitrarily classified as follows in order to provide a descriptive framework: A) Medium-velocity gating, with mean residence times in each level in the order of several tens of ms (Fig. 1A). B) An often-encountered behavior: residence in a predominant substate is interrupted by frequent, very brief (in this example complete or almost complete) closures (Fig. 1B). C) An additional fast gating between two states superimposed on B-type behavior (Fig. 1C,D). D) Bursts of fast gating between levels, including (Fig. 1F) or not (Fig. 1E) the predominant one. E) prolonged fast gating between two states (Fig. 1G). Fast gating was also observed in planar bilayer experiments with yeast mitochondrial outer membrane vesicles (not shown).

Another observation to be emphasized is that VDAC could close completely, at least when in a single-channel situation. As illustrated in Fig. 2, closures were generally brief and occurred from conductance substates and in the context of fast gating. Such behavior would be difficult to recognize in a multi-channel situation. Fig. 3 shows that longer-lasting complete closures could take place in the presence of König's polyanion (KPA), a co-polimer of methacrylate, maleate and styrene (35) which has long been known to decrease current conduction by VDAC (but not to induce complete closures, until this report) (27,36). When the polyanion was present on one side of the planar membrane only, the phenomenon depended on the polarity of the applied voltage, occurring when it was negative on the side of KPA. KPA has also been reported to inhibit (27,35) or even to block (37) the flux of ATP through the pore.

The return of the channels to the completely open state upon switching the applied voltage to zero was always rapid, but in many cases the reopening was sufficiently delayed to be observable. In these experiments the presence of a transmembrane salt concentration gradient made it possible to observe current conduction at zero applied voltage thanks to the selectivity properties of the channel. Fig. 4 shows one such case. Upon switching the *cis*-side voltage from +40 to 0 mV, a positive current was flowing in the circuit, indicating that the channel had an overall cationic selectivity. An opening event occurred after 160 ms; the current

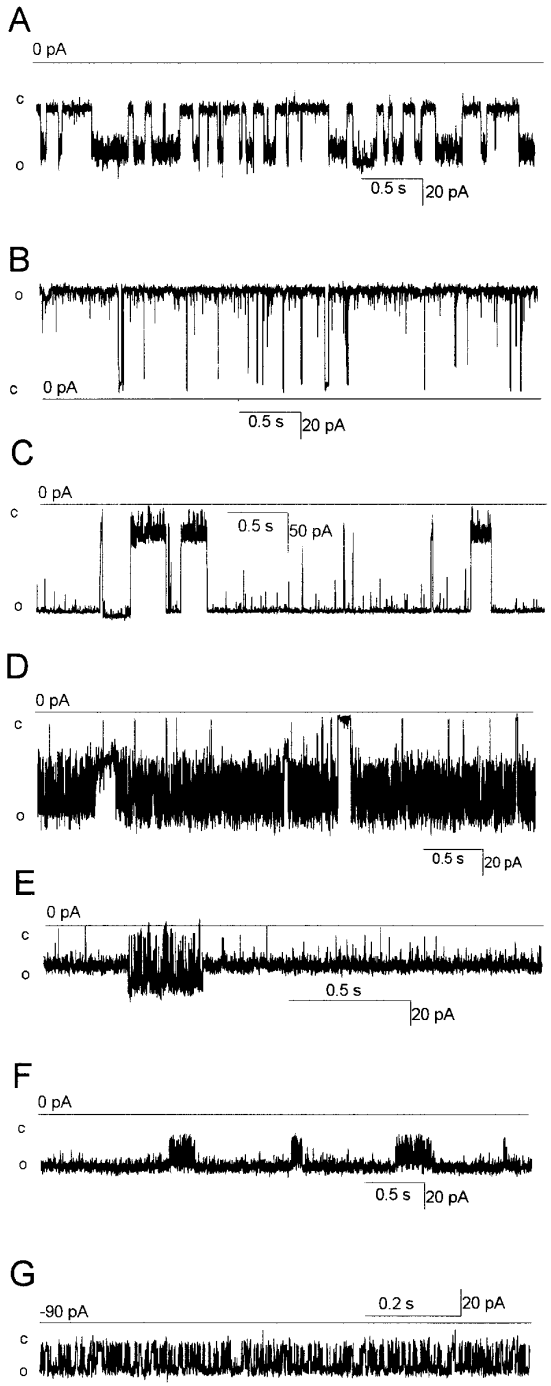

FIG. 1. Representative examples of fast gating by single porin channels. Symmetrical 1 M KCl medium. (A) Vcis: −40mV. State 1: conductance: 1.11 nS; mean residence time (m.r.t): 127ms; time constant (τ) of the exponential fitting the time distribution histogram: 102 ms. State 2: 1.83 nS; m.r.t.: 68 ms; τ: 55 ms. (B) Vcis: +40mV. State 1: 195 pS (possibly a leak conductance); m.r.t.: 0.59 ms; τ: 0.52 ms. State 2: 2.0 nS; m.r.t. and τ: 6 ms. (C) Vcis: −40 mV. Main state: 3.25 nS. Transitions to rapid gating between approx. 0.63 and 1.27 nS, with nearly complete closures as well. (D) Vcis: −50 mV. The channel gated between a 68 pS conductance state (possibly a leak conductance, state 1) and a fast-gating state with an approximately 1.8 nS max. conductance (state 2). State 1: m.r.t.: 26 ms; τ: 13 ms. State 2: m.r.t.: 487 ms; τ: 399 ms. While in state 2, the channel gated between approx. 770 pS (level 1) and 1.8 nS (level 2) conductance levels. Level 1: m.r.t.: 0.8 ms; τ: 0.5 ms. Level 2: m.r.t.: 1.0 ms; τ: 0.9 ms. (E) Vcis: −40 mV. Main conductance level: 870 pS. Bursting between approx. 1.3 nS and a completely closed state. (F) Vcis: −40 mV. main conductance level: 1 nS. Bursts of fast gating to an approx. 0.5 nS conductance level. (G) Vcis: −60 mV. State 1: 1.7 nS. m.r.t.: 1.5 ms; τ: 1.2 ms. State 2: 2.1 nS. m.r.t.: 2.4 ms; τ: 1.9 ms. In this particular experiment the channel exhibited this gating mode for prolonged periods, returning repeatedly to it after switching to other modes.

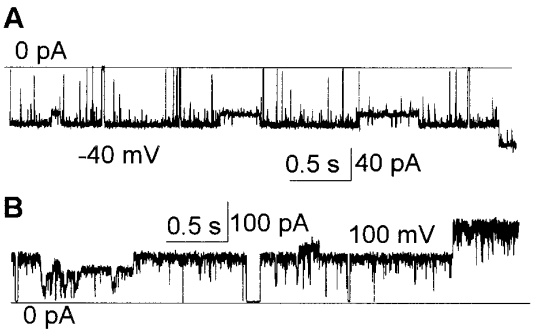

FIG. 2. The porin can close completely. Exemplificative traces from the same one-channel experiment in symmetrical 1 M KCl at Vcis −40 (A) and +100 (B) mV. See also Fig. 1.

flowing afterwards had a negative sign, indicating that a transition to anion selectivity and to the fully open state had taken place.

In our hands the voltage dependence of the porin also differed from the accepted canon, in that the response to voltage jumps was somewhat asymmetrical. As we have already reported (10), both the extent of current decrease and the kinetics with which a steady state was eventually reached differed depending on the polarity of the voltage applied (not shown, see 10). Furthermore, as already reported by Mirzabekov et al. (30), the prolonged application of high voltage often resulted in the porin assuming a "permanent" low-conductance state which it would not abandon upon application of zero voltage (not shown, see 30).

DISCUSSION

While its structure has not yet been solved, VDAC is universally believed to be a β-barrel pore. Modeling studies have led to 16 (38, 39) or to 12 (40) β-strand structures, plus an α-helical N-terminal, presumably protruding into the cytosol in vivo (38, 41). The pore is a cylinder with an approximately circular cross-section and a diameter of approximately 3 nm (42). The mass of the protein is such that only a thin layer of polypeptide separates the lumen of the pore and the membrane lipids (19). This deceptively simple structure gives rise to a surprisingly varied electrophysiological behavior. A large variety of voltage-favored partially closed

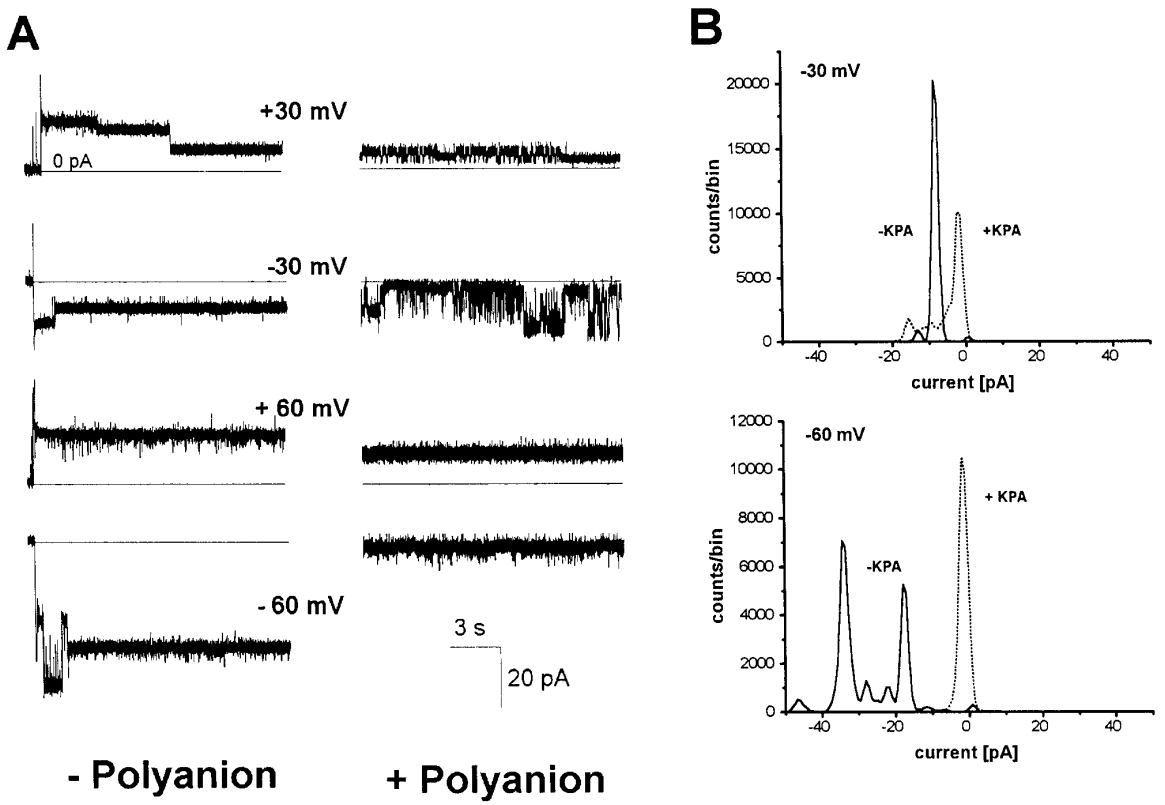

FIG. 3. The presence of KPA can induce long-lasting complete closures. Exemplificative traces (A) and corresponding current amplitude histograms (B) at the indicated *cis* voltages. 100 mM symmetrical KCl. KPA (0.4 mg/ml) was added only into the *cis* chamber.

states exist, as witnessed not only by the multiple conductances and selectivities displayed, but also by the inactivating effect of prolonged exposure to high voltage (19, 30; our observations, not shown). The fact that the switch to the fully open state can occur with a measurable delay upon returning to zero voltage (Fig. 4) indicates the presence of a non-zero energy barrier for this process. The dependence of voltage-induced closure on the polarity of the applied potential adds complexity. Interconversion between states can take place with a great variety of kinetic modes (Fig. 1). A dynamic model aiming to explain this complexity must be correspondingly complex. Major structural rearrangement upon gating has been indicated by a number of studies (43-46). Colombini and coworkers e.g. (19, 26) have proposed a gating model whereby a sector of the cylindrical pore wall would "slide out" of the membrane, moving to the negative-voltage side, thus leading to a narrowing of the pore. Opening would correspond to re-insertion of the displaced strands. This model seems insufficient to explain the variety of conductances (including zero) and the very fast kinetics. More than one gating mechanism is probably required to fully account for the observations. The major Gram-negative porins (OmpF and OmpC) also display both slow and fast gating modes, voltage-dependent reversible closure and inactivation upon prolonged application of voltage (47-49). One likely possibility (39) is therefore that VDAC conforms to the folding pattern adopted by bacterial porins of known structure (50-53; refs. therein), in which the N-terminal segment loops partially into the lumen of the pore, acting as a "plug". Small variations in the degree of penetration of this loop into the channel could account for part of the many substates. Furthermore, such short-range motions would be expected to require only very short times, thus explaining the fast kinetics.

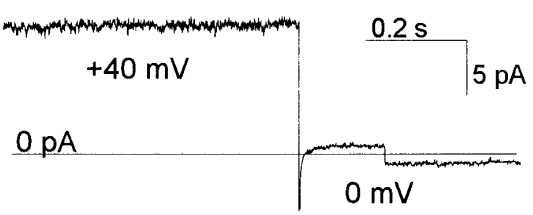

FIG. 4. Delayed re-opening at 0 mV. Exemplificative current trace recorded in the presence of salt gradient (390 mM KCl on *cis* side and 100 mM KCl on *trans* side). Following the change of voltage from +40 mV to 0 mV VDAC exhibited first cationic (for 160 ms) and then anionic selectivity.

Our observations also strongly suggest that the conformational changes of VDAC are influenced by inter-

actions with other copies of the porin: slow gating is predominantly observed if the bilayer contains more than one VDAC molecule, whereas fast transitions predominate when only one copy is present. VDAC can certainly interact with copies of itself as well as with a number of other proteins: insertion in a bilayer is strongly facilitated if at least one molecule is already present (25, 26); dimeric and trimeric homocomplexes of VDAC have been reported (54, 55). An involvement of the N-terminal segment in these interactions would be consistent with the indications that it protrudes from the membrane (38, 41) and with its suspected role in determining fast gating. When engaged in intermolecular contact, the loop would be prevented from acting as a "plug". Electrophysiological recordings have provided evidence in favor of interactions between OmpF/OmpC trimers (48). Matching residue-frequency motifs have recently been found in VDAC and bacterial porins (56). A benzodiazepine receptor is present in *Rhodobacter capsulatus* and *Rhodobacter spheroides* and it comprises porin (57, 58). These observations also suggest a structural (and evolutionary) relationship between the bacterial and mitochondrial pore proteins.

One problem associated with the localization of porin in non-mitochondrial membranes is that the pore must be tightly regulated, i.e., kept completely closed most of the time, lest the cells die. Current understanding of VDAC gating however holds that the pore does not close completely. Closure can be enhanced by a soluble proteic factor (59-61) and by polyanions, including KPA, but both classes of effectors are reportedly only capable of increasing the steepness of the voltage dependence and/or induce lower-conductance (but still conductive) states. We report here that VDAC can instead close completely even at moderate applied voltages, both on its own (Fig. 3) and in the presence of KPA (Fig. 4), at least when observed as a single channel in a membrane. This finding removes a conceptual difficulty in accepting the localization of porin in cellular membranes. We also note that the patterns of electrical activity revealed by patch-clamp investigations of the mitochondrial outer membrane (62-64) did not match expectations based on the behavior of reconstituted VDAC. Evidence has been presented (65) that the permeability of the outer mitochondrial membrane is lower than would be expected based on the assumption that all its porin molecules are in the fully open state.

Both biochemical and electrophysiological data thus indicate that VDAC, far from being a simple "sieve", is a functionally complex, highly regulated, multiple-purpose pore.

ACKNOWLEDGMENTS

The authors thank Prof. D. Wolff for initial help with the bilayer technique and Drs. C. Cola and T. Starc for performing some experiments. The stay of Dr. Gy. Bàthori in Padova was financed by a long-term postdoctoral Telethon fellowship. Dr. I. Schmèhl is grateful for the Magyary Zoltàn postdoctoral fellowship. Dr. I. Szabò is a recipient of a postdoctoral fellowship of the University of Padova. The authors are especially grateful to Telethon-Italy for financial support (Grants A.44 and A.59).

REFERENCES

1. Thinnes, F. P. (1992) *J. Bioenerg. Biomembr.* **24,** 71–75.
2. Shoshan-Barmatz, V., Hadad, N., Feng, W., Shafir, I., Orr, I., Varsanyi, M., and Heilmeyer, L. M. G. (1996) *FEBS Lett.* **386,** 205–210.
3. Junankar, P. R., Dulhunty, A. F., Curtis, S. M., Pace, S. M., and Thinnes, F. P. (1995) *J. Muscle Res. Cell Motil.* **16,** 595–610.
4. McEnery, M. W., Snowman, A. M., Trifiletti, R. R., and Snyder, S. H. (1991) *Proc. Natl. Acad. Sci. USA* **89,** 3170–3174.
5. Kinally, K. W., Zorov, D. B., Antonenko, Y. N., Snyder, S. H., McEnery, M. W., and Tedeschi, H. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1374–1378.
6. Brdiczka, D. (1991) *Biochim. Biophys. Acta* **1071,** 291–312.
7. Zoratti, M., and Szabo, I. (1995) *Biochem. Biophys. Acta* **1241,** 139–176.
8. Zoratti, M., Szabo, I., and DePinto, V. (1994) *in* Molecular Biology of Mitochondrial Transport Systems (Forte, M., and Colombini, M. Eds.), pp. 153–168, Springer-Verlag, Heidelberg.
9. Szabò, I., and Zoratti, M. (1993) *FEBS Lett.* **330,** 201–205.
10. Szabò, I., De Pinto, V., and Zoratti, M. (1993) *FEBS Lett.* **330,** 206–210.
11. Beutner, G., Ruck, A., Riede, B., Welte, W., and Brdiczka, D. (1996) *FEBS Lett.* **396,** 189–195.
12. Kroemer, G., Zamzami, N., and Susin, S. A. (1997) *Immunol. Today* **18,** 44–51.
13. Bernardi, P. (1996) *Biochem. Biophys. Acta* **1275,** 5–9.
14. Hong Yu, W., Wolfgang, W., and Forte, M. (1995) *J. Biol. Chem.* **270,** 13998–14006.
15. Jakob, C., Gotz, H., Hellmann, T., Hellmann, K. P., Reymann, S., Florke, H., Thinnes, F. P., and Hilschmann, N. (1995) *FEBS Lett.* **368,** 5–9.
16. Dermietzel, R., Hwang, T. K., Buettner, R., Hofer, A., Dotzler, E., Kremer, M., Deutzmann, R., Thinnes, F. P., Fishman, G. I., Spray, D. C., and Siemen, D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 499–503.
17. Benz, R., Maier, E., Thinnes, F. P., Gotz, H., and Hilschmann, N. (1992) *Biol. Chem. Hoppe-Seyler* **373,** 295–303.
18. Benz, R. (1994) *Biochim. Biophys. Acta* **1197,** 167–196.
19. Colombini, M. (1989) *J. Membr. Biol.* **111,** 103–111.
20. Colombini, M. (1994) *Curr. Top. Membr.* **42,** 73–101.
21. Colombini, M., Blachly-Dyson, E., and Forte, M. (1996) *in* Ion Channels (Narahashi, T., Ed.), Vol. 4. pp. 169–202, Plenum Press, New York.
22. Thomas, L., Kocsis, E., Colombini, M., Erbe, E., Trus, B. L., and Steven, A. C. (1991) *J. Structural Biol.* **106,** 161–171.
23. Peng, S., Blachly-Dyson, E., Forte, M., and Colombini, M. (1992) *J. Bioenerg. Biomembr.* **24,** 27–31.
24. Xu, X., and Colombini, M. (1996) *J. Biol. Chem* **271,** 23675–23682.
25. Xu, X., and Colombini, M. (1997) *Biophys. J.* **72,** 2129–2136.
26. Zizi, M., Thomas, L., Blachly-Dyson, E., Forte, M., and Colombini, M. (1995) *J. Membr. Biol.* **144,** 121–129.
27. Benz, R., and Brdiczka, D. (1992) *J. Bioenerg. Biomembr.* **24,** 33–39.

28. Rostovtseva, T., and Colombini, M. (1996) *J. Biol. Chem.* **271,** 28006–28008.
29. Rostovtseva, T., and Colombini, M. (1997) *Biophys. J.* **72,** 1954–1962.
30. Mirzabekov, T., Ballarin, C., Nicoloni, M., Zatta, P., and Sorgato, M. C. (1993) *J. Membr. Biol.* **133,** 129–143.
31. Elkeles, A., Breiman, A., and Zizi, M. (1997) *J. Biol. Chem.* **272,** 6252–6260.
32. DePinto, V., Prezioso, G., and Palmieri, F. (1987) *Biochim. Biophys. Acta* **905,** 499–502.
33. DePinto, V., Benz, R., and Palmieri, F. (1989) *Eur. J. Biochem.* **183,** 179–187.
34. Alvarez, O. (1986) *in* Ion Channel Reconstitution (Miller, C. Ed.), pp.115–130, Plenum Publishing Corp. New York.
35. Konig, T., Kocsis, B., Meszaros, L., Nahm, K., Zoltan, S., and Horvath, I. (1977) *Biochim. Biophys. Acta* **462,** 380–389.
36. Colombini, M., Yeung, C. L., Tung, J., and Konig, T. (1987) *Biochim. Biophys. Acta* **905,** 279–286.
37. Bathori, G., Sahin-Toth, M., Fonyo, A., and Ligeti, E. (1993) *Biochim. Biophys. Acta* **1145,** 168–176.
38. De Pinto, V., Prezioso, G., Thinnes, F., Link, T. A., and Palmieri, F. (1991) *Biochemistry* **30,** 10191–10200.
39. Rauch, G., and Moran, O. (1994) *Biochem. Biophys. Res. Comm.* **200,** 908–915.
40. Blachly-Dyson, E., Peng, S. Z., Colombini, M., and Forte, M. (1990) *Science* **247,** 1233–1236.
41. De Pinto, V., and Palmieri, F. (1992) *J. Bioenerg. Biomembr.* **24,** 21–26.
42. Guo, X. W., Smith, P. R., Cognon, B., D'Arcangelis, D., Dolginova, E., and Mannella, C. (1995) *J. Struct. Biol.* **114,** 41–59.
43. Zimmerberg, J., and Parsegian, A. (1986) *Nature* **323,** 36–39.
44. Zimmerberg, J., and Parsegian, A. (1987) *J. Bioenerg. Biomembr.* **19,** 351–358.
45. Mirzabekov, T. A., and Ermishkin, L. N. (1989) *FEBS Lett.* **249,** 375–378.
46. Ermishkin, L. N., and Mirzabekov, T. A. (1990) *Biochim. Biophys. Acta* **1021,** 161–168.
47. Delcour, A. H., Adler, J., and Kung, C. (1991) *J. Membr. Biol.* **119,** 267–275.
48. Berrier, C., Coulombe, A., Houssin, C., and Ghazi, A. (1992) *FEBS Lett.* **306,** 251–256.
49. Buehler, L. K., and Rosenbusch, J. P. (1993) *Biochem. Biophys. Res. Comm.* **190,** 624–629.
50. Schulz, G. E. (1994) *in* Bacterial Cell Wall (Ghuysen, J.-M., and Hakenbeck, R., Eds.), pp. 343–352, Elsevier, Amsterdam.
51. Cowan, S., and Schirmer, T. (1994) *in* Bacterial Cell Wall (Ghuysen, J.-M., and Hakenbeck, R., Eds.), pp. 353–362, Elsevier, Amsterdam.
52. Schirmer, T., Keller, T. A., Wang, Y.-F., and Rosenbusch, J. P. (1995) *Science* **267,** 512–514.
53. Meyer, J. E. W., Hofnung, M., and Schulz, G. E. (1997) *J. Mol. Biol.* **266,** 761–775.
54. Linden, M., and Gellerfors, P. (1983) *Biochim. Biophys. Acta* **736,** 125–129.
55. Krause, J., Hay, R., Kowollik, C., and Brdiczka, D. (1986) *Biochim. Biophys. Acta* **860,** 690–698.
56. Mannella, C. A., Neuwald, A. F., and Lawrence, C. E. (1996) *J. Bioenerg. Biomembr.* **28,** 163–169.
57. Yeliseev, A. A., and Kaplan, S. (1995) *J. Biol. Chem.* **270,** 21167–21175.
58. Yeliseev, A. A., Krueger, K. E., and Kaplan, S. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 5101–5106.
59. Holden, M. J., and Colombini, M. (1988) *FEBS Lett.* **241,** 105–109.
60. Holden, M. J., and Colombini, M. (1993) *Biochim. Biophys. Acta* **1144,** 396–402.
61. Liu, M. Y., and Colombini, M. (1992) *J. Bioenerg. Biomembr.* **24,** 41–46.
62. Tedeschi, H., Mannella, C. A., and Bowman, C. L. (1987) *J. Membr. Biol.* **97,** 21–29.
63. Moran, O., Sciancalepore, M., Sandri, G., Panfili, E., Bassi, R., Ballarin, C., and Sorgato, M. C. (1992) *Eur. Biophys. J.* **20,** 311–319.
64. Sorgato, M. C., and Moran, O. (1993) *Crit. Revs. Biochem. Mol. Biol.* **18,** 127–171.
65. Saks, V. A., Vasil'eva, E., Belikova, Yu, O., Kuznetsov, A. V., Lyapina, S., Petrova, L., and Perov, N. A. (1993) *Biochim. Biophys. Acta* **1144,** 134–148.