Is the mammalian porin channel, VDAC, a perfect cylinder in the high conductance state?

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Abstract The mammalian porin channel (VDAC, porin-31BM) was reconstituted in planar lipid bilayers under voltage clamp conditions. The radii of both entrances of the channel were examined using a method that consisted in filling the channel with different non-electrolytes through its *cis* or *trans* entrances while recording single channel conductances. As a result it was found that the geometry of channels formed by porin-31BM could not be approximated by a perfectly cylindrical pore. In fact there is an asymmetry in the geometry of the channel: the diameters of the *cis* and *trans* entrances were estimated to be ~2 nm and ~4 nm respectively.

Key words: Voltage-dependent anion-selective channel; Ion channel; Size

1. Introduction

The voltage-dependent anion-selective channel (VDAC) is formed by a 31 kDa protein and has been found in mitochondrial and cytoplasmic membranes of several species (for reviews see [1,2]). The polypeptide chain of VDAC from different sources is usually composed of 282–283 amino acids [3–8]. The sequence is characterized by numerous segments of alternating polar/non-polar residues and structural analysis using computer-based programs predicts the existence of 12–16 β-strands and a small α-helix at the N-terminus. Although the primary sequence is not well conserved, this amphipathic α-helical structure is preserved in organisms as distantly related as man and *Saccharomyces cerevisiae* or *Neurospora crassa* [5,9].

Based on available data about the secondary structure of VDAC and on the evidence that a single molecule of VDAC is sufficient to form a transmembrane channel [10], working models of VDAC channels were proposed. All of them model the open channel as a perfectly cylindrical pore whose walls are constituted by β-strands, with or without the participation of the α-helix [9,11–13]. Since knowledge about the geometry of ion channels is extremely relevant for our understanding of their mode of action we decided to examine if the lumen of the VDAC pore actually has a cylindrical geometry. A recently developed methodology [14] was used to establish the diameters of both entrances of the bovine muscle porin channel (porin-31BM) and to get additional information about the geometry of the channel lumen.

2. Materials and methods

Porin isolated from bovine muscle was a generous gift from Dr. Thinnes (Max-Plank Institute fur Experimentelle Medizin, Göttingen, Germany). The protein was obtained using methodology described elsewhere [6] for porin from human tissue. Pure phosphatidylcholine (type V-E) and cholesterol were purchased from Sigma. The non-electrolytes (NE) polyethylene glycol (PEG) 300 and PEG 400 (Sigma), PEG 600 (Riedel de Haen), PEG 1000 and PEG 1450 (Sigma), PEG 2000, PEG 3000 and PEG 4000 (Loba Chema), PEG 8000 and PEG 12000 (Sigma) were used. Hydrodynamic radii of NE, taken from [15–17], were as follows: 0.6±0.03 nm PEG 300; 0.7±0.03 nm PEG 400; 0.8±0.04 nm PEG 600; 0.94±0.03 nm PEG 1000; 1.05±0.03 nm PEG 1450; 1.22±0.03 nm PEG 2000; 1.44±0.03 nm PEG 3000; 1.92±0.03 nm PEG 4000; 2.1±0.03 nm PEG 4600; 3.05±0.03 nm PEG 8000; and 3.75±0.03 nm PEG 12000. Other chemicals used were of analytical grade.

Twice-distilled water was used to prepare all buffer solutions. The standard solution used in the bilayer experiments contained 1.15 M KCl and 5 mM HEPES and the pH was adjusted to 7.0 with 1.0 M KOH. In the experiments carried out to determine channel size this solution also contained 20% (w/v) of an appropriate NE. The conductivity of each buffer solution was measured with a HI 9033 (Hanna Instruments) multi-range conductivity meter at 25°C.

Bilayer lipid membranes were formed by the method of Mueller et al. [18] from 2% phosphatidylcholine in *n*-decane. Measurement of electrical parameters of black lipid membranes was done as described elsewhere [19]. Porin obtained from bovine muscle was added to the *cis* compartment to a final concentration of ~4 ng/ml and the bilayer was clamped at 10 mV. The *trans* compartment of the experimental chamber was connected to virtual ground. The record was discarded if any of the open channels temporarily closed. Current fluctuations were measured by hand and single channel conductances were estimated by dividing the single channel current by the voltage imposed through the membrane. The mean value of the main pool of the channel conductance was used for following analysis. The relatively small amounts of lower and higher conductance steps were not considered in this mean value.

Channel sizing experiments were done as recently described [14]. Briefly, this method allowed us to determine the size of each entrance of the bovine muscle porin channel (porin-31BM) and to obtain additional information about the geometry of the channel lumen by measuring the conductance of the channel with a different NE in the *cis* and *trans* compartments of the bilayer. In this study the side of the channel which protrudes from the plane of the membrane into the side of porin-31BM addition is defined as the *cis* entrance and the other side is the *trans* entrance of the channel. To determine the size of each channel entrance, a solution containing an impermeant NE is added to one side of the bilayer while solutions containing NEs of different radii are added to the other side. For instance, if we want to determine the diameter of the *cis* entrance we add PEG 4600 to the *trans* chamber (we previously determined that PEG 4600 does not enter the channel from either side; see [19]) and measure single channel con-

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ductances while adding NE of different radii to the *cis* chamber. As long as the NE is able to enter the channel from the *cis* entrance and fill it, even partially, the value for single channel conductance measured will be smaller than the control value obtained without NE addition. When the NE is no longer able to penetrate the channel from its *cis* entrance, single channel conductance returns to its control value and the dimension of the entrance can be estimated from the NE radius. The same procedure can then be applied to measure the *trans* entrance. These two maneuvers will give us the conductance of a single ion channel in the presence of the test NE in the *cis* (g_i^{cis}) or in the *trans* (g_i^{trans}) entrance of the channel.

3. Results and discussion

As a first approach, if the channel lumen behaves as a perfect cylinder we would expect g_i^{cis} to have a value close to g_i^{trans}. In contrast, if g_i^{cis} and g_i^{trans} are different from each other this suggests not only that the geometry is not perfectly cylindrical but that the channel entrances have different sizes. Table 1 shows the values of single channel conductances for the porin-31BM channel obtained using different NEs in the two chambers of the bilayer. For comparison the published data [19] on porin-31BM channel conductance in the presence of the same NE at both entrances of the channel (g_i^{both}) are included. Analyzing the data we observe that the values of g_i^{trans} are almost always smaller than g_i^{cis}. This difference can result from asymmetries in the geometry of the channel. These data can also be directly used to determine the apparent sizes of the channel entrances by an analysis of the relationship between g^i and the NE's hydrodynamic radii. However, to get a more precise measurement of the channel radius one should analyze the dependence of filling of the channel with NE on the hydrodynamic radius of the NE. As shown elsewhere [14] the filling parameter (F) can be determined using the following relationship:

$$F = [(g_o - g_i)/g_i]/[(\chi_0 - \chi_i)/\chi_i]$$

where g_o is the single channel conductance in the presence of an impermeant NE or the single channel conductance in a solution without NE; g_i is the single channel conductance in the presence of a solution containing 20% (w/v) of a NE with access to the channel interior from both sides or from only one side; χ_o is the conductivity of the solution without NE or the conductivity of the virtual volume free of NE in a solution with NE; χ_i is the conductivity of the solution containing 20% (w/v) of a given NE.

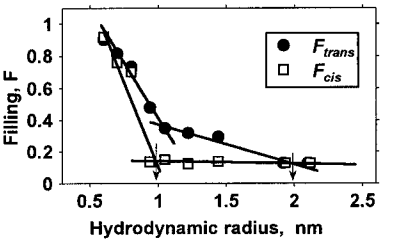

Fig. 1. The dependence of F^{cis} (□) and F^{trans} (●) on the hydrodynamic radii of non-electrolytes. The values of F^{cis} and F^{trans} for each NE were calculated as described in the text. The lines are a least squares fit to the experimental points. Values of the parameters in the presence of PEG 1000, PEG 1450, PEG 2000, PEG 3000, PEG 4000 and PEG 4600 (for F^{cis}) and in the presence of PEG 4000 and PEG 4600 (for F^{trans}) were used to draw the horizontal line. To build the ramp lines the data obtained in the presence of NE with radii ranging from 0.6 to 0.94 nm were used for F^{cis} and from 0.6 to 1.05 nm and from 1.05 to 2.1 nm for F^{trans}. Standard deviation was equal to or smaller than the width of the symbols used. Arrows indicate the values of the radii of porin-31BM channel entrances. All other experimental conditions are as described in the text and Section 2.

The results obtained by measuring single channel conductances and conductivities and applying these values to calculate filling parameters (F) are shown in Fig. 1. When the channel was filled from its *cis* entrance (F^{cis}), considerable (up to 90%) filling was observed in the presence of PEG 300, PEG 400 and PEG 600. In contrast, a PEG with a slightly larger molecular mass and hydrodynamic size (PEG 1000) does not enter the channel. As expected, molecules with radii larger than PEG 1000 also did not enter the channel from the *cis* entrance ($F^{cis} \sim 0$). Therefore, we can assume that the limiting size for the radius of the *cis* entrance of the channel should not exceed 0.9 nm.

The filling of the channel from its *trans* entrance (F^{trans}) was found to be considerably different, as illustrated in Fig. 1. Only molecules with radii (r) equal to or larger than that of PEG 4000 ($r = 1.92$ nm) were not able to penetrate the channel. Therefore, we can assume that the limiting size for the

Table 1
Conductances of the porin-31BM channel

Substance	r (nm)	χ (mS/cm)	g_o^{both}	g_o^{trans}	g_o^{cis}
1. Control		132.0	4.66 ± 0.2 (212)		
2. PEG 300	0.60	65.4	2.38 ± 0.3 (152)	2.43 ± 0.3 (140)	2.43 ± 0.2 (170)
3. PEG 400	0.70	62.9	2.18 ± 0.3 (146)	2.46 ± 0.3 (138)	2.54 ± 0.2 (178)
4. PEG 600	0.80	65.2	2.48 ± 0.3 (104)	2.66 ± 0.2 (110)	2.67 ± 0.3 (208)
5. PEG 1000	0.94	61.4	3.03 ± 0.3 (181)	3.00 ± 0.3 (155)	4.03 ± 0.4 (193)
6. PEG 1450	1.05	63.4	3.22 ± 0.4 (98)	3.38 ± 0.3 (121)	4.01 ± 0.4 (163)
7. PEG 2000	1.22	63.3	3.3 ± 0.4 (87)	3.46 ± 0.2 (118)	4.11 ± 0.3 (91)
8. PEG 3000	1.44	66.2	3.76 ± 0.4 (118)	3.60 ± 0.2 (116)	4.10 ± 0.2 (43)
9. PEG 4000	1.92	62.2	4.07 ± 0.4 (131)	4.07 ± 0.4 (72)	4.07 ± 0.4 (41)
10. PEG 4600	2.10	61.5	4.06 ± 0.3 (125)		
11. PEG 8000	3.05	61.9	4.06 ± 0.3 (85)		
12. PEG 12000	3.75	62.2	4.26 ± 0.3 (54)		

Single channel conductances (nS) are expressed as mean ± S.D. The numbers in parentheses indicate the numbers of single channel events counted in the main pool, which represented more than 60% of all the events observed in a given condition. g^{both} was obtained in the presence of the same NE on both sides of the bilayer; g^{cis} was measured in the presence of a given NE on the *cis* side of the bilayer while PEG 4600 was present on the *trans* side and g^{trans} refers to the conductance measured in the presence of a given NE on the *trans* side of the bilayer while PEG 4600 was present at the *cis* side. χ (mS/cm) is the conductivity of the solutions. r (nm) is the hydrodynamic radius of non-electrolytes. Other conditions are as described in Section 2 and in the text.

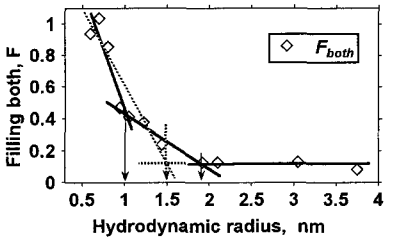

Fig. 2. Dependence of the parameter F^{both} on the hydrodynamic radius of non-electrolytes. Values of the parameter F in the presence of PEG 4000, PEG 4600, PEG 8000 and PEG 12000 were used to draw the horizontal line. Arrows at the extremities indicate the values of the radii of porin-31BM channel entrances. The arrow at the middle indicates the 'mean' radius of the channel, when F values for NE that penetrate the channel are fitted by a single straight line (dotted line). All other experimental conditions are as described in the text and in the legend to Fig. 1.

radius of the *trans* entrance of the channel should not exceed 1.9 nm. This value is about two times larger than the radius of the *cis* entrance, implying that the geometry of the VDAC channel might be conical. However, the existence of two slopes on the F^{trans}-r relation suggests that the geometry of the lumen of the channel from the *trans* to the *cis* entrance is not smooth. More important, although the conical geometry decreases the discrepancy between measured and calculated conductances for the VDAC channel (as compared to models assuming cylindrical geometry), it still does not solve it; the measured conductance is 4.66 nS while that calculated assuming that the pore is a perfect cone (with length 6 nm) is 12.4 nS. Therefore the channel, if conical, must have constrictions in its lumen that further reduce its conductance and might explain the two slopes in the F^{trans}-r relation.

Since our measurements using different NEs in the entrances of the channel disclosed significant asymmetry in the channel geometry we decided to re-analyze the data previously published with the same NE in both chambers of the bilayer [19] using the physically defined filling parameter instead of the empirical permeability parameter suggested earlier [16,17]. Fig. 2 shows the result of this analysis. The dependence of F^{both} on r is very similar to that shown for F^{trans} in Fig. 1 and can also be fitted by two straight lines with different slopes: a steeper one for $0.6 \leq r \leq 1.05$ and a shallower one for $1.05 \leq r \leq 1.92$. Fitting the relation using only one straight line (dashed line in Fig. 2) results in a channel radius of ~ 1.5 nm, in close agreement with the value previously published using the permeability parameter [19]. However, the use of the two straight lines to fit the relation is totally justified on statistical grounds: using the method of least squares we find a value of 0.047 for two lines versus 0.084 for a single line. Interestingly, the two straight lines that best fit the F-r relation intersect at a radius of ~ 1 nm, close to the radius of the smaller entrance to the channel, while the second straight line and the line parallel to the r axis (which represents NEs that cannot penetrate the channel from either side) intersect at a radius of ~ 1.9 nm, corresponding to the radius of the larger entrance to the channel.

In conclusion, the established difference in the size of the entrances of the porin-31BM channel is clear evidence that a cylindrical geometry is not adequate to model the functional channel. Furthermore, even a simple conical geometry is not appropriate, suggesting that the geometry of the porin-31BM channel should be more complex.

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References

[1] Benz, R. (1994) Biochim. Biophys. Acta 1197, 167–196.
[2] Mannella, C.A., Forte, M. and Colombini, M. (1992) J. Bioenerg. Biomembr. 24, 7–19.
[3] Michara, K. and Sato, R. (1985) EMBO J. 4, 769–774.
[4] Kleene, R., Pfanner, N., Pfaller, R., Link, T.A., Sebald, W., Neupert, W. and Tropschug, M. (1987) EMBO J. 6, 2627–2633.
[5] Kayser, H., Kratzin, H.D., Thinnes, F.P., Götz, H., Schmidt, W.E., Eckart, K. and Hilschmann, N. (1989) Biol. Chem. Hoppe-Seyler 370, 1265–1278.
[6] Jürgens, L., Ilsemann, P., Kratzin, H.D., Hesse, D., Eckart, K., Thinnes, F.P. and Hilschmann, N. (1991) Biol. Chem. Hoppe-Seyler 372, 455–463.
[7] Ha, H., Hajec, P., Bedwell, D.M. and Burrows, P.D. (1993) J. Biol. Chem. 268, 12143–12149.
[8] 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.
[9] De Pinto, V., Prezioso, G., Thinnes, F.P., Link, T.A. and Palmieri, F. (1991) Biochemistry 30, 10191–10206.
[10] Peng, S., Blachly-Dyson, E., Colombini, M. and Forte, M. (1992) J. Bioenerg. Biomembr. 24, 27–31.
[11] Forte, M., Guy, H.R. and Mannella, C.A. (1987) J. Bioenerg. Biomembr. 19, 341–350.
[12] Blachly-Dyson, E., Peng, S., Colombini, M. and Forte, M. (1989) J. Bioenerg. Biomembr. 21, 471–483.
[13] Blachly-Dyson, E., Peng, S., Colombini, M. and Forte, M. (1990) Science 247, 1233–1236.
[14] Krasilnikov, O.V., Yuldasheva, L.N., Cruz, J.B. and Nogueira, R.A. (1996) Med. Microbiol. Immunol. 185, 112.
[15] Krasilnikov, O.V., Sabirov, R.Z. and Ternovsky, V.I. (1991) Proteins, Ionic Channels and Regulation of Ion Transport Through Membranes, FAN, Tashkent.
[16] Krasilnikov, O.V., Sabirov, R.Z., Ternovsky, V.I., Merzlyak, P.G. and Muratkhodjaev, J.N. (1992) FEMS Microbiol. Immunol. 105, 93–100.
[17] Sabirov, R.Z., Krasilnikov, O.V., Ternovsky, V.I. and Merzlyak, P.G. (1993) Gen. Physiol. Biophys. 12, 95–111.
[18] Mueller, P., Rudin, D.O., Tien, H.T. and Wescott, W.C. (1963) J. Phys. Chem. 67, 534–535.
[19] Krasilnikov, O.V., Carneiro, C.M.M., Yuldasheva, L.N., Campos-de-Carvalho, A.C. and Nogueira, R.A. (1996) Braz. J. Med. Biol. Res. 29, 1691–1697.