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1/*f*-Noise of Open Bacterial Porin Channels

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Abstract. General diffusion pores and specific porin channels from outer membranes of gram-negative bacteria were reconstituted into lipid bilayer membranes. The current noise of the channels was investigated for the different porins in the open state and in the ligand-induced closed state using fast Fourier transformation. The open channel noise exhibited 1/*f*-noise for frequencies up to 200 Hz. The 1/*f*-noise was investigated using the *Hooge* formula (Hooge, *Phys. Lett.* **29A:** 139–140 (1969)), and the *Hooge* parameter α was calculated for all bacterial porins used in this study. The 1/*f*-noise was in part caused by slow inactivation and activation of porin channels. However, when care was taken that during the noise measurement no opening or closing of porin channels occurred, the *Hooge* Parameter α was a meaningful number for a given channel. A linear relationship was observed between α and the single-channel conductance, *g*, of the different porins. This linear relation between single-channel conductance and the *Hooge* parameter α could be qualitatively explained by assuming that the passing of an ion through a bacterial porin channel is—to a certain extent—influenced by nonlinear effects between channel wall and passing ion.

Key words: Noise analysis — Porin channels — Gram-negative bacteria — Outer membrane — Lipid bilayer membrane

Introduction

The outer membranes of gram-negative bacteria act as molecular filters with defined exclusion limits for hydrophilic solutes (Benz, 1994). Responsible for these molecular sieving properties are bacterial porins, major classes of outer membrane proteins that form water-filled hollow cylinders (Weiss et al., 1991; Cowan et al., 1992; Schirmer et al., 1995). In contrast to voltage- or ligand-gated ion channels in nerve and muscle tissues that contain many α-helices, the porin channels are formed almost entirely from 16 or 18 amphipathic β-strands (Weiss et al., 1991; Schirmer et al., 1995). Furthermore, bacterial porin channels are predominantly in the open configuration and close only at high voltages above 100 mV (Lakey, 1987) or when a specific porin is occupied by a substrate molecule, for which it has a binding site inside the channels (Luckey & Nikaido, 1980; Benz, 1988; Maier et al., 1988). Substrate-specific porins represent a major advantage for the efficient scavenging of substrates at very small concentrations (Benz, 1988; Andersen, Jordy & Benz, 1995).

In recent publications we have investigated the current noise of the LamB-channel of *Escherichia coli* outer membrane reconstituted into lipid bilayer membranes (Nekolla, Andersen & Benz, 1994; Andersen et al., 1995). This maltose- and maltooligosaccharide-specific porin shows high current noise of the Lorentzian type in the presence of sugars without any indication of cooperativity between the monomers within a trimer. The corner frequencies of the power density spectra have been used for the evaluation of sugar binding kinetics to the binding site inside the channel and for the calculation of the sugar transport through the LamB-channels of *E. coli* and *Salmonella typhimurium* (Nekolla et al., 1994; Andersen et al., 1995; Jordy et al., 1996). The evaluation of the sugar-induced current noise has been similar to that of the ameloride-induced current noise of the Na^+ channels in frog skin (Lindemann, 1980). The open LamB-channels show 1/*f*-noise, which is probably in part caused by slow opening and closing of the LamB-channels (Nekolla et al., 1994) because its amplitude varies from membrane to membrane. Nevertheless, the 1/*f*-noise was present in all experiments even when opening and closing of channels is minimized.

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1/*f*-noise, also known as *Flicker Noise,* can be found in many physical and biological systems (Hooge, 1969, 1972; Hooge & Gaal, 1971; Verveen & DeFelice, 1974; Conti & Wanke, 1975; DeFelice, 1981; Sigworth, Urry & Prasard, 1987; Bezrukov & Vodyanoy, 1994). It is often encountered in semiconductor devices (electrical current over a semiconductor usually exhibits 1/*f*-fluctuations), but also in very complex systems which might be governed by nonlinear dynamics (Bak & Creutz, 1994). The intensity of earthquakes, for example, is usually proportional to some power of 1/*f*, if *f* describes the number of earthquakes with a certain intensity during a period of time (Bak & Chen, 1991). In membrane systems, 1/*f*-noise has been found for the current flow through the dimerized gramicidin channel inserted into lipid bilayer membrane (Sauvé & Bamberg, 1978). On the other hand, careful analysis of the current noise of the open gramicidin channel has not provided any evidence for the existence of 1/*f*-noise (Sigworth et al., 1987).

Many attempts have been made to explain 1/*f*-behavior of current noise through electronic devices and membranes. Although there does not exist a good explanation for the occurrence of the *Flicker Noise,* Hooge (1969) has developed a formula, which allowed the analysis of the results obtained from noise experiments on the basis of a single constant, the parameter α. It is the number to which the spectral amplitude of the frequency spectrum is directly proportional. For semiconductor devices α was estimated to have a value around 10^{-4} (Hooge, 1991). However, for ion transport through biological and artificial membranes it was not clear whether 1/*f*-noise really existed in these systems or whether the observed 1/*f*-noise simply reflected the slow shift of the systems or the superposition of several Lorentzians (Sauvé & Bamberg, 1978; Sauvé & Szabo, 1985; Sigworth et al., 1987).

In this study we investigated the power density spectra of current noise of a variety of bacterial porin channels. These channels are particularly well suited for such a study because they are permanently open and because they possess a wide scope of single-channel conductance, which ranges in this study between 10 pS and 4 nS for a single conductive unit in 1 M KCl (Benz, 1994). The results are discussed under the assumption that the ions passing to the channels interact with the internal loop inside the porin channels.

Materials and Methods

Purification of the Different Porins

Purification of LamB

LamB was isolated from envelopes of maltose-grown cells of *E. coli* TK24, which lacks OmpC, OmpF, and OmpA. Details of the isolation procedure have been described in detail elsewhere (Vos-Scheperkeuter, Hofnung & Witholt, 1984).

Purification of Tsx

Tsx was isolated and purified from *E. coli* strain CH8, which is a derivative of the Tsx-overproducing strain P400 and lacks, or is severely deficient in, the major outer membrane proteins OmpA, OmpC, OmpF, and LamB (Maier et al., 1988).

Purification of TolC

TolC was isolated from envelopes of the *E. coli* strain KS 26, which lacks the outer membrane proteins OmpC, OmpF, and LamB (Schülein et al., 1991) and contained the plasmid pAX 629 to increase the concentration of TolC in the outer membrane. TolC was purified as has been described previously (Benz et al., 1993*b*).

Purification of OmpC

OmpC from *E. coli* K12 was isolated and purified from strain RAM 105 (Misra and Benson, 1988; K. Bauer and R. Benz, *unpublished results*).

Purification of OprP

OprP from *Pseudomonas aeruginosa* was isolated and purified according to an established procedure (Siehnel et al., 1992).

Purification of ScrY

ScrY was isolated from envelopes of the *E. coli* strain KS 26, which lacks the outer membrane proteins OmpC, OmpF, and LamB and contains the multicopy plasmid pPSO28-37 (Schülein, Schmid & Benz, 1991).

Purification of porin from Roseobacter denitrificans

Porin from the phototrophic gram-negative bacteria *Roseobacter denitrificans* was isolated and purified as has been described previously (Neumann et al., 1995).

PorB porin from Neisseria

PorB porin from *Neisseria gonorrhoeae* VPI was isolated as described previously (Rudel et al., 1996). It was a kind gift of Thomas Rudel, Max-Planck-Institut für Biologie, Tübingen, Germany.

Lipid Bilayer Experiments and Noise Analysis

Black lipid bilayer membranes were formed as described previously (Benz et al., 1978). The instrumentation consisted of a Teflon chamber with two aqueous compartments connected by a small circular hole. The hole had a surface area of 0.2 mm^2. Membranes were formed by painting onto the hole a 1% solution of diphytanoyl phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) in n-decane. The KCl solutions (Merck, Darmstadt, F.R.G.) were used unbuffered and had a pH around 6. The porins were reconstituted into the lipid bilayer membranes by adding the concentrated stock solution to the aqueous phase bathing a membrane in the black state. Except stated otherwise the temperature was maintained at (22 ± 1)°C throughout. The noise

analysis was performed as has been described previously (Nekolla et al., 1994; Andersen et al., 1995; Jordy et al., 1996). In brief, the membrane current was measured with a pair of calomel electrodes with salt bridges switched in series with a battery-operated voltage source and a current amplifier (Keithley 427 with a four-pole filter). The feedback resistors of the current amplifier were between 0.01 and 10 GΩ. For control purposes the amplified signal was monitored with a strip chart recorder (Rikadenki). This allowed the detection of the insertion of channels other than those under investigation in the membranes because it was possible to suppress the channel conductance to a large extent. The amplified AC-component of the signal was analyzed with a digital signal analyzer (Ono Sokki CF 210), which performed fast Fourier transformation of the current noise. The spectra were composed of 400 points and they were averaged 128 times. The further analysis of the power density spectra was performed with an IBM compatible computer with a 486 processor. The Fourier transformed data were transferred into the computer as simple text files (two columns of numbers, corresponding to x-y-values) and evaluated with the program EASYPLOT (© by Spiral Software, Brookline, MA).

Theoretical Background

1/*f*-Noise and the Hooge Formula

An empirical relation for the explanation of the power density spectra of 1/*f*-noise in physical systems is given by *Hooge's* formula (Hooge, 1969; Hooge & Gaal, 1971):

$$S(f) = \frac{\alpha \cdot I_O^2}{N \cdot f}. \qquad (1)$$

S(f) is the amplitude of the power density spectrum at a given frequency *f*, I_O is the current flow through the open pores, *N* is the number of open channels in a membrane, and α is the so-called *Hooge* parameter. Since bacterial porin trimers contain three individual channels, *N* is the number of on-steps observed in experiments with lipid bilayer membranes (which corresponds to the reconstitution of trimers into the membranes) times three (Benz, 1994).

Results

Current Noise of Unmodified Lipid Bilayer Membranes

In a first set of experimental conditions we investigated the power density spectra of lipid bilayer membranes in the absence of porins. Membranes were formed of diphytanoyl phosphatidylcholine/n-decane in 1 M KCl solution. After the membrane had reached the black state the spectral density of the current noise of the membrane was measured to assure that the membrane had not an unusually high conductance, which could produce excess noise. The measurements were only continued when the specific conductance of the membrane was about 10 nS/cm^2 or less. Figure 1*A* and *B* show the power density spectrum of such a control experiment. It is noteworthy that the power density spectra of these experiments were very similar to those known from the literature (Kolb, Läuger & Bamberg, 1975; Sauvé & Bamberg, 1978). In particular, the spectral density was very small for the frequency range between 1 and 200 Hz (*see* Fig. 1*B*). This is the frequency range that was of interest for the study of the 1/*f*-noise. At higher frequencies the noise increased because of the intrinsic noise of the preamplifier that produces a current noise through the membrane capacity.

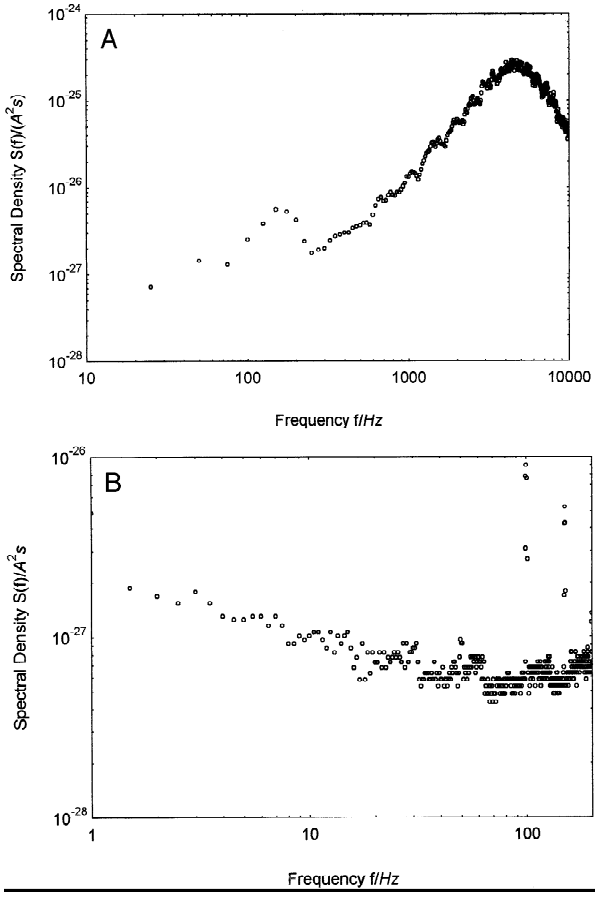

Fig. 1. Power density spectra of a diphytanoyl phosphatidylcholine/*n*-decane membrane bathed in 1 M KCl. Panel *A* shows the power density spectra between 10 and 10,000 Hz. The bandwidth of the preamplifier was 3 kHz. Panel *B* shows the power density spectrum of the same membrane between 1 and 200 Hz. The applied membrane potential was 20 mV; $T = 25$°C.

1/*f*-Noise of Open Porin Channels

When the control experiments were completed we added the porins in small concentrations (5 to 500 ng/ml). One

to two minutes after the addition of the proteins the membrane conductance started to increase in a stepwise fashion caused by the formation of ion-permeable channels. The conductance of the individual porin channels was taken from these recordings and the average single-channel conductance was calculated from the current steps. It was compared to that, which has been derived previously from the initial characterization of the bacterial porins used in this study. It is noteworthy that the single-channel conductance derived here were identical to the previous results. Furthermore, the standard deviations of the single-channel distributions were similar to those that have been observed previously. The final increase was orders of magnitude above that in the absence of porins (about 10 nS/cm^2) and reached values of 1 to 100 μS/cm^2 within about 20 to 30 min after addition of the porins (Benz, Ishii & Nakae, 1980). About 30 min after the addition of the porins the membrane current at a voltage of 20 mV was approximately stationary and it was possible to start the measurement of the current noise. It is noteworthy, however, that the membrane current had to be carefully checked for further insertion of channels and for their slow inactivation at high current resolution. The reason for this was that both processes may contribute to the power density spectra and tend to increase the 1/*f*-noise as we have shown in a recent publication (Nekolla et al., 1994).

Noise measurements were performed only for periods of time, in which we did not observe any channel opening or closure at very high current resolution. Furthermore, the spectra were averaged 128 times, which means that the contribution of occasional channel opening and closing to the noise spectra, if its occurrence was not detected, was minimized in particular at high channel densities (>100 channels). At smaller channel densities it was easy to be detected in the simultaneous single-channel recordings. This means that the possibility of major contributions to current noise due to opening and closing channels during the 1/*f*-measurements can be excluded. Figure 2 shows the power density spectra of the current noise obtained for four different bacterial porins at a membrane potential of 20 mV. Except for experiments with the Tsx-channel (because of its small single-channel conductance) the current noise of the membranes without porin was not subtracted because of its small amplitude in the considered frequency range. The frequency range for which 1/*f*-noise was observed differed usually for different membranes because of the diverse number of reconstituted channels in a given membrane, but rarely exceeded 200 Hz. At higher frequencies the power density spectrum started to increase because of the intrinsic noise of the preamplifier that produced a current noise through the membrane capacitance. The number of channels was calculated from their single-channel conductance measured in this study and

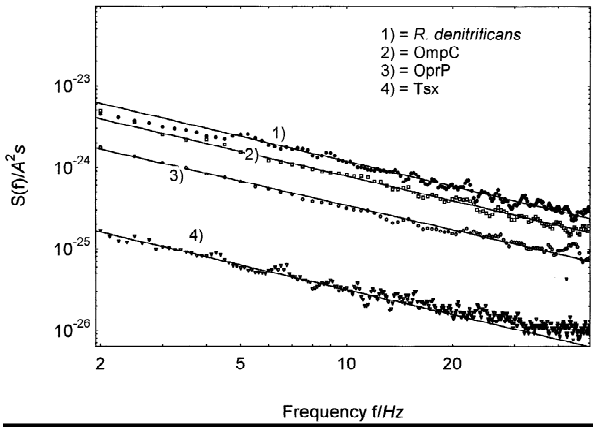

Fig. 2. Power density spectra of diphytanoyl phosphatidylcholine/*n*-decane membranes containing (i) 180 *Roseobacter denitrificans* porin-channels, (ii) 190 OmpC-channels from *Escherichia coli,* (iii) 200 OprP-channels from *Pseudomonas aeruginosa* and (iv) 220 Tsx-channels from *Escherichia coli*. The number of channels were calculated from the membrane conductance and the single-channel conductance of the corresponding porins in 1 M KCl and was multiplied by three because of the three channels in a trimer. The single-channel conductance were 4 nS (porin from *Roseobacter denitrificans*), 1.3 nS (OmpC from *Escherichia coli*), 250 pS (OprP from *Pseudomonas aeruginosa*) and 10 pS (Tsx from *Escherichia coli*). The aqueous phase contained 1 M KCl. The applied membrane potential was 20 mV, T = 22°C. The straight lines show least squares fits of the power density spectra with the equation $S(f) = D/f^c$.

earlier investigations (Benz, 1994), which always showed excellent agreement. Since the porin trimers contain three individual channels (Weiss et al., 1991; Cowan et al., 1992; Schirmer et al., 1995) the number of trimers were multiplied by the factor three.

Evaluation of the Hooge Parameter α for Current Noise Through Porin Channels of OprP

Double logarithmic plots of the power density spectra of open channel noise from the phosphate specific diffusion pore of OprP were fitted to the equation $S(f) = D/f^c$ ($D = S(1Hz)$) by using a least squares fit. The power densities were usually not exactly proportional to 1/*f*, but to some power of 1/*f* with exponents that varied between 0.9 and 1.1 (*see* Table 2). In the ideal case of $c = 1$ this should result in a straight line with slope −1 in a double logarithmic plot. From *Hooge's* formula one can calculate the parameter α according to (Hooge, 1969; Hooge & Gaal, 1971):

$$S(1Hz) \cdot f: = D = \frac{\alpha \cdot I_O^2}{N}, \qquad (2)$$

and α is given by (D, accordingly, has the dimension A^2):

$$\alpha = \frac{N \cdot D}{I_O^2}. \quad (3)$$

The experiments suggested that the 1/*f* noise component created by the passage of current through the reconstituted porin-channels of OprP was proportional to the number of channels because the ratio *S*(*1Hz*)/*N* showed only minor variations. This could also be seen from the data of Table 1, which gives the number of reconstituted channels, *N*, the power density of the 1/*f* component, *S*(*1Hz*), at a frequency of 1 *Hz*, the ratio *S*(*1Hz*)/*N*, and the parameter α derived according to Eq. (3) for 11 different membranes in which different numbers of OprP channels were reconstituted (*see* also Discussion). Table 1 shows that α was fairly constant ($\alpha = (3.122 \pm 0.567) \cdot 10^{-3}$) and that the power of the plot of *S*(*f*) versus *f* was close to minus one ($c = 1.033 \pm 0.010$). The single-channel conductance of a single conductive unit of the OprP porin was 83.3 pS for the monomer in a trimer (Benz & Hancock, 1987).

Evaluation of the Hooge Parameter α for Other Porins

We performed also with a variety of other porins from gram-negative bacteria measurements of the current noise and examined the 1/*f*-noise. These were the following porins: LamB (specific for maltose and maltooligosaccharides (Benz et al., 1986)), ScrY (specific for sucrose (Schülein et al., 1991)), OprP (specific for anions in particular for phosphate (Benz, Egli & Hancock, 1993*a*)), Tsx (specific for nucleosides (Benz et al., 1988)), TolC (specific for small peptides and involved in the export of proteins out of *E. coli* (Benz et al., 1993*b*)) and the general diffusion porins *Roseobacter denitrificans* (Neumann et al., 1995), OmpC from *E. coli* (Benz, 1988) and PorB from *Neisseria gonorrhoeae* (Rudel et al., 1996). For each of these porins we calculated *Hooge's* parameter α from the power density spectra by using plots similar to those shown in Fig. 2. The results of these measurements are summarized in Table 2, which contains besides α also the single-channel conductance (for the single conductive unit in a trimer) of these porins in 1 M KCl together with the standard deviation of the single-channel distribution and the slope of the fit of the power density spectra. For most of the porins the fit of the data using Eq. (3) was satisfactory. Only in the case of Tsx larger standard deviations were obtained, probably because of the very small single-channel conductance and the rather small contribution of the 1/*f*-component to the total power density spectra. It is noteworthy that the *Hooge* parameter calculated for the open alamethicin channel ($\alpha \leqslant 10^{-5}$), which has a conductance similar to OmpC or PorB is approximately in the same range as measured here for these bacterial porin channels (Bezrukov & Vodyanoy, 1991).

Lorentzian Type of Noise of Specific Porins

It is noteworthy that some of the specific porins exhibited Lorentzian type of noise, when the corresponding substrates were added to the aqueous phase. This was the case of LamB of *E. coli* and of ScrY from *S. typhimurium.* The reason for this is the existence of a binding site inside the channel, which leads to a dose-dependent block of ion transport through the channels (Schülein et al., 1991; Nekolla et al., 1994; Andersen et al., 1995). In control experiments with LamB, we could demonstrate that the binding of substrate was still possible when the measurement of the 1/*f*-noise was completed. The analysis of the Lorentzian type of current noise of these experiments yielded the rate constants (± SD) for the binding of maltotriose. These were $1.12 \cdot 10^7$ l/(mol · sec) (± 0.05) for the on-rate constant (k_1), 4960 (± 204) l/sec for the off-rate constant (k_{-1}), 2260 (± 10) l/mol for the stability constant of maltotriose binding, and $g = 56$ pS for the single-channel conductance. These results agreed reasonably well with the constants that have been published recently (Andersen et al., 1995). This suggested that both 1/*f*-noise and Lorentzian type of noise are intrinsic properties of the LamB-channel. The current noise of the other specific porins changed also when the substrates were added to the aqueous phase. However, we observed in these cases preferentially white noise, which indicated that the kinetics of substrate binding was too fast to be resolved properly in our experiments.

Discussion

The experimental results presented here suggest that 1/*f*-noise exists in open bacterial porin channels and that its spectral density is correlated to the single-channel conductance of the individual channels. This means that the *Hooge* parameter α is dependent on their single-channel conductance. The current noise through the open porin channels contained within the frequency range between 1 and 200 Hz a strong 1/*f*-noise-component in the power density spectra. There exist several possibilities for the explanation of the 1/*f*-noise, which will be discussed in some detail in this study that are: (i) Noise because of translational or rotational movement of the porin channels within the plane of the membrane, (ii) noise because of the superposition of several Lorentzians caused by the independent closing and opening of channel, and (iii) noise because of nonlinear (chaotic) effects within the channels.

Table 1. Parameters of the 1/*f* current noise through open OprP-channels from *Pseudomonas aeruginosa* outer membrane[a]

Experiment	I_0/pA	N	c	$\frac{S(1Hz)}{10^{-24}\,A^2}$	$\frac{S(1Hz)}{N \cdot 10^{-26}\,A^2}$	$\alpha/10^{-3}$
JUL21M01	,65	13	1.032	0.8261	6.355	7.626
JUL21M03	,85	17	1.015	0.7466	4.392	5.270
JUL21M06	195	39	1.039	0.7992	2.049	2.459
JUL21M15	570	114	0.948	1.918	1.682	2.019
JUL26M14	1150	230	0.992	3.791	1.648	1.978
JUL26M16	1650	330	1.001	4.908	1.487	1.785
JUL28M06	5200	1040	0.961	20.48	1.969	2.363
JUL28M09	5700	1140	1.001	27.61	2.422	2.906
JUL28M11	6100	1220	1.044	37.13	3.043	3.652
AUG02M11	970	194	1.006	4.040	2.082	2.499
AUG11M08	1125	225	0.989	3.337	1.483	1.780
Mean ± SD			1.033± 0.010		2.061± 0.435	3.122± 0.567

[a] The membranes were formed from diphytanoyl phosphatidylcholine/*n*-decane. The aqueous phase contained 1 M KCl and between 10 and 50 ng/ml OprP. The membrane potential was 20 mV; T = 21–22°C. I_0 is the current through the open channels and N is their number calculated with a single-channel conductance of 250 pS (corresponding to 83.3 pS for a single conductive unit in a trimer (Benz & Hancock, 1987)). c is the power of the fit Eq. $S(f) = D/f^c$, D is the power density of the 1/*f* noise at 1 Hz and $\alpha = N \cdot D/I_0^2$ is the fit parameter of the 1/*f* noise using *Hooge*'s formula (Hooge, 1969).

Table 2. Parameters of the 1/*f* current noise through different porins from gram-negative bacteria[a]

Porin	g/pS	c	$\alpha/10^{-4}$
General diffusion pores:			
OmpC (*E. coli* K12)	433 ± 65	0.981 ± 0.022	2.04 ± 0.45
PorB (*N. gonorrhoeae*)	1000 ± 85	1.007 ± 0.014	0.432 ± 0.033
R. denitrificans	1333 ± 97	0.971 ± 0.055	0.324 ± 0.032
Specific porins:			
LamB (*E. coli*)	50 ± 7.5	1.011 ± 0.019	22.1 ± 4.4
Scr Y (*Salmonella typhimurium*)	470 ± 55	0.995 ± 0.040	4.47 ± 0.10
OprP (*Pseudomonas aeruginosa*)	83 ± 6.5	0.990 ± 0.010	31.2 ± 5.7
Tsx (*E. coli*)	3 ± 0.8	0.974 ± 0.021	265 ± 118
TolC (*E. coli*)	27 ± 1.9	0.934 ± 0.084	247 ± 45

[a] g is the single-channel conductance ± SD of a unit channel (i.e., of a porin monomer), and c is the slope of the plot of the power density spectra of the current noise as a function of the frequency. α ± SD is the *Hooge* factor calculated from Eq. (3).

Pore Diffusion Within the Membrane

In a recent paper Bezrukov and Vodyanoy (1994) have attempted to explain the 1/*f* current noise of channels reconstituted into lipid bilayer membranes. The authors suggest that lateral or rotational diffusion of pores in the membrane could be the cause for 1/*f*-noise. Membranes represent fluid two-dimensional arrays of lipid and protein and it is possible that channel-forming proteins move along the lipid structure with a given diffusion constant. Using special assumptions for the dependence of the current, $i(x)$, on its location, x within the membrane, it has been demonstrated that the spectral density of the current noise is proportional to $1/f^a$(Bezrukov & Vodyanoy, 1994). These assumptions are either that the current $i(x)$ through the channel is a rectangular function of its location x, which means that the slope a of the spectral density in a double logarithmic plot is equal to 3/2. When the current $i(x)$ through the channel is proportional to $1/\sqrt{|x|}$ the slope a is equal to 1 (Bezrukov & Vodyanoy, 1994). In both cases it has been suggested that frequency ranges could be expected, in which the spectral density is proportional to 1/*f*. Furthermore, in these cases the spectral density $S(f)$ should be a direct function of the lateral diffusion coefficient of the channel, provided that the diffusion is sufficiently slow, which can be expected for a protein in the plane of a membrane.

The comparison of the results of our experiments with the formalism proposed by Bezrukov and Vodyanoy (1994) makes it rather unlikely that the lateral diffusion of the porin trimers could account for our data.

In particular, *Hooge's* parameter α varied considerably for the different porins. The highest value was obtained for the specific porins Tsx and TolC, while the smallest value for α was found for the general diffusion porin PorB from *N. gonorrhoeae.* Between both extremes, α varies about 500-fold. The molecular mass of outer membrane porins including TolC (52 kDa, Benz et al., 1993*b*) ranges between 30 kDa and 50 kDa (corresponding to 90 to 150 kDa for the trimer; Benz & Bauer, 1988), and PorB and TolC have a similar secondary structures and are both in the trimeric form, which means that we would expect only minor differences if any for their lateral diffusion coefficients in a lipid bilayer membrane. These considerations make it rather unlikely that lateral channel diffusion can account for the dependence of the spectral density of the current noise on 1/*f*.

Superposition of Lorentzian-shaped Power Density Spectra Due to Switching Channels

Another system, in which a dependence of the current noise on 1/*f* has been found is the head-to-head dimerized gramicidin channel (Sauvé & Bamberg, 1978). Later on it has been argued that the 1/*f* behavior of the current noise in this system does not represent the intrinsic property of the open gramicidin channel but represents the fast switching of channels between the open and the closed states as the origin of 1/*f*-noise (Sauvé & Szabo, 1985; Ring, 1986; Sigworth et al., 1987). We have to admit that a similar explanation is also possible for the 1/*f*-noise of the open porin channels and we have recently argued in favor of such a superposition of Lorentzians to explain the variation of *Hooge's* parameter α for the LamB-channel (Nekolla et al., 1994). However, in this study we carefully controlled the current during the noise experiments and we subjected only those parts of the current recordings to noise analysis, in which we did not observe any opening or closing of channels at high current resolution. As a consequence, the *Hooge* parameter α was virtually constant for a given porin, despite large variations of the number of reconstituted channels in the different reconstitution experiments with the same channel-forming protein. Thus, the superposition of Lorentzians caused by opening and closing of channels cannot account for the 1/*f*-noise of the porin channels.

Hooge's Parameter α is a Function of the Single-Channel Conductance of the Porins

Plotting the different average values for α vs. the single-channel conductance, *g*, yielded a quite astonishing result. Obviously, α decreased for increasing *g*. This can be seen from Fig. 3, which shows a double logarithmic plot of *Hooge's* parameter α as a function of the single-channel conductance, *g*. A least squares fit of $\alpha(g)$ had the form $\alpha = \kappa/g^b$ with a slope $b = 1.16$ on the double logarithmic scales. This means in a first approximation that α is roughly proportional to *1/g*. Following Eq. (3) one can write:

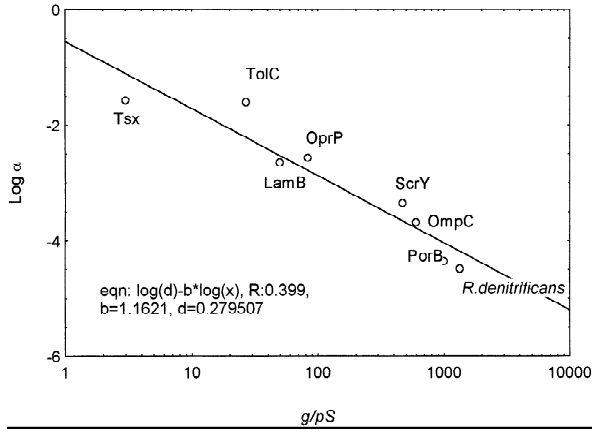

Fig. 3. Plot of the *Hooge*-Parameter α as a function of the single channel conductance *g* of different bacterial porin monomers. The data were taken from Table 2. The straight line corresponds to a least squares fit of the data with a slope $b = 1.1621$.

$$D = \frac{\alpha \cdot I_O^2}{N} = \alpha \cdot N \cdot g^2 \cdot U^2 \tag{4}$$

with $I_O = N \cdot g \cdot U$, where U is the membrane voltage. Our experimental observations suggest that α is not a constant, but it is roughly proportional to *1/g* for different porin channels. This means that it can be written as $\alpha \equiv \kappa/g$ with κ being a new factor, whose relations to other parameters of the systems are unknown at present. Thus, Eq. (4) can be rewritten as:

$$D = \kappa \cdot N \cdot g \cdot U^2 = \kappa \frac{N^2 g^2 U^2}{Ng} = \kappa \frac{I_O^2}{G} = \kappa \cdot I_O \cdot U. \tag{5}$$

The constant κ has a value of $2.80 \cdot 10^{-13}$ *S* (Fig. 3). However, it is not clear if it should be understood as a new more universal constant because a similar complete study with other systems is still lacking.

At that point the question arises, which parameters determine the size of *g* of the porin channels. In a first order approximation the porin channels can be treated as a simple resistance *R* expressed as $R = \rho \cdot l/A$ with *l* the length of a channel, *A* its average cross-section and ρ the specific resistance. However, X-ray crystallography of porins has demonstrated that this picture is too simple as Fig. 4 clearly demonstrates (Weiss et al., 1991; Cowan et al., 1992; Schirmer et al., 1995). Porin channels are formed by 16 to 18 amphipathic transmembrane

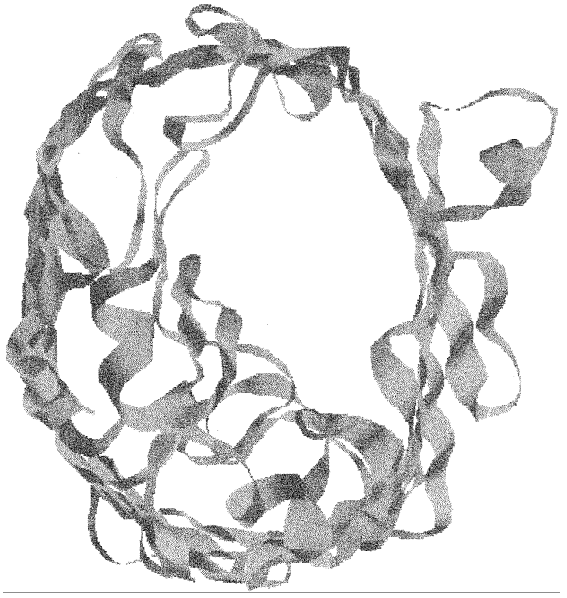

Fig. 4. Simplified structure of an OmpF monomer seen perpendicular to the outer membrane surface. The channel is formed by a β-barrel cylinder. The central friction of the channel is formed by the third external loop from the *N*-terminal end that is folded inside the cylinder. The data were taken from Cowan et al., 1992.

β-strands that form a hollow cylinder. The size of the cylinder is restricted by the third external loop (localized between the β-strands 5 and 6 from the N-terminal end) that folds inside the channel (Figure 4). The effective diameter of *E. coli* general diffusion porins is about 0.8 × 1.0 nm (Cowan et al., 1992) and that of LamB is even smaller (Schirmer et al., 1995). This means that the conductance of the porins depends strongly on the size of the loop and on the local electric field created by the amino acids localized within the channel. In channels with high single-channel conductance the ions passing the channel do not interact strongly with the channel walls and the loop 3 because of their large diameter and their energetically favorable conformation.

The 1/*f*-noise increased with decreasing single-channel conductance of the bacterial porins, which is limited by the friction inside the channel. When we consider the known 3-D structure of porins it is possible the 1/*f*-noise is caused by the interaction between the third external loop localized inside the β-barrel channel and the ions that have to pass the loop on the way through the channel. The noise is on the other hand most likely not created by some sort of ''channel breathing,'' which has been discussed in detail elsewhere (Sigworth, 1985) and which should create Lorentzian noise. This means that we are probably not dealing here with the moving of the loop between one or several defined positions. The mechanism of the creation of the 1/*f*-noise in the porin channels may be similar as has been discussed by Bak and Chen (1991) and Bak and Creutz (1994) for the *Self-Organized Criticality* (*SOC*) of the sand avalanche system in a two dimensional lattice. It has been shown for such a system that 1/*f*-noise can be interpreted as a superposition of avalanches in an SOC-state (Bak & Chen, 1991; Christensen, Fogedby & Jensen, 1991; Bak & Creutz, 1994), for which it has been shown that the power density spectra are given under certain conditions by:

$$S(f) \propto f^{-1-\mu}, \qquad \text{with } -1 < \mu < 1. \tag{6}$$

This could mean for the porin system discussed here that ions do not penetrate the pores regularly, but each single channel can be looked at as a system in a critical state. Thus, ion diffusion across the pores takes place in ion avalanches of different sizes. Since the porin channels consist of complicated structures, each ion crossing the membrane may leave a pore in a slightly different state and its conformation is changed after every event. This could be compared with the sandpile model, in which every grain ''sees'' slightly different conditions when it is dropped on the lattice. The total 1/*f*-noise is in this case a sum of the 1/*f*-noises of all single pores. Taking these considerations together, it might well be that *Hooge's* parameter, α, can be considered as a measure for interactions between the channel interior, in particular with external loop 3 that folds inside the β-barrel cylinder and provides major interaction with the penetrating ions. Such a loop does not exist in the open gramicidin A channels, which are long and narrow. This may be the reason that 1/*f*-noise is not observed in this system (Sigworth et al., 1987).

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