Examining Noise Sources at the Single-Molecule Level: $1/f$ Noise of an Open Maltoporin Channel

Sergey M. Bezrukov[1,2] and Mathias Winterhalter[3,4]
[1]Laboratory of Physical and Structural Biology, NICHD, NIH, Bethesda, Maryland 20892-0924
[2]St. Petersburg Nuclear Physics Institute, Gatchina, Russia 188350
[3]Department of Biophysical Chemistry, Biozentrum, Basel, Switzerland
[4]IPBS-CNRS, Toulouse, France
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We have studied the phenomenological origin of $1/f$ noise in a solute-specific bacterial ion channel, maltoporin. We show that after excision of small, but resolvable stepwise changes in the recordings of the current through a single open channel, the $1/f$ noise component disappears and the channel exhibits noise that is "white" below 100 Hz. Combined with results of a recent noise study of several bacterial porins, our observations suggest that $1/f$ noise is caused by the equilibrium conductance fluctuations related to the conformational flexibility of the channel pore structural constituents.

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The origin of $1/f$ noise in biological membranes is a long-standing problem that has been attracting significant interest for more than 30 years. Since Derksen and Verveen published their pioneering study [1] of $1/f$ (flicker) noise in a node of Ranvier, a large number of different mechanisms of its generation were suggested (for a short recent review, see Ref. [2]). While a single well-accepted explanation of this phenomenon does not exist, researchers generally agree that $1/f$ noise is related to channel conductance fluctuations seen as open-closed transitions with a complex dynamics [3–5]. Indeed, ion channels in their open state can be remarkably free of $1/f$ noise [6,7].

Recently, $1/f$ noise was found and discussed in a series of studies performed on different bacterial porin channels reconstituted into planar lipid bilayers [8–12]. Multichannel membranes were used in these experiments. It was concluded that the noise was generated by *open* channels because it was present even in the steady recordings where the absence of additional channel insertions or channel closures was carefully controlled. The $1/f$ noise was attributed [11] to a nonequilibrium critical state of the channel, where ion diffusion across the pore happens in differently sized "avalanches" of self-organized criticality [13].

In this short communication we report results of noise analysis performed on a single channel reconstituted into planar lipid bilayers. Maltoporin is a specific diffusion channel from the outer cell wall of the bacteria *Escherichia coli* that serves as an efficient transporter for maltooligosaccharides. The structure of the maltoporin channel has been determined to a resolution of 3.1 Å [14]. The maltoporin complex forms three water-filled channels per trimer, the functional unit of the proteins in the outer membrane. In each monomer, eighteen β-strands span the outer membrane and form a barrel with short turns at the periplasmic side and large loops at the outside of the cell. Several of the outer loops are packed inside the channel. The third loop, L3, is especially important; it folds into the barrel to form a constriction zone at half the height of the channel. This loop contributes significantly to channel permeability properties defining its conductance for small ions and exclusion limits for larger solutes.

We show that the maltose-specific channel, maltoporin, in its open state switches between substates of slightly differing conductance. Occasionally, spontaneous closures of one of the monomers in the channel trimer are also observed. These stepwise conductance transitions are only weakly voltage dependent, which suggests their equilibrium nature. We find that the channel is virtually $1/f$-noise free in any of the substates and $1/f$ noise is generated by random transitions between them. These transitions do not lead to the complete channel closure and, therefore, are not resolvable in multichannel experiments. When the stepwise current jumps are excluded, the channel noise is white at frequencies below 100 Hz. We conclude that these small, equilibrium, "second-order" conformational transitions together with more rare occasional monomer closures are responsible for the $1/f$ noise measured in multichannel experiments.

Figure 1 presents a typical recording of the current through a planar lipid bilayer that was filtered using 2 ms averaging interval. It shows that after spontaneous insertion of a single maltoporin channel, its conductance fluctuates between substates differing by several pS. Figure 1 insets show these fluctuations on a finer scale. Channel inserts in a substate of 183 pS conductance, stays in this substate for about $1/2$ s, and then goes to a substate of 187 pS where it spends about 1 s. The process of channel spontaneous transitions continues through a relatively long (about 4 s) substate of 184 pS. It is seen that transitions happen in a stepwise manner and have a wide spread of characteristic times. In addition, rare reversible events of complete closure of one of the monomers in the porin trimer (see below) are observed.

We have analyzed the power spectral density of current noise of the open maltoporin channel using two strategies.

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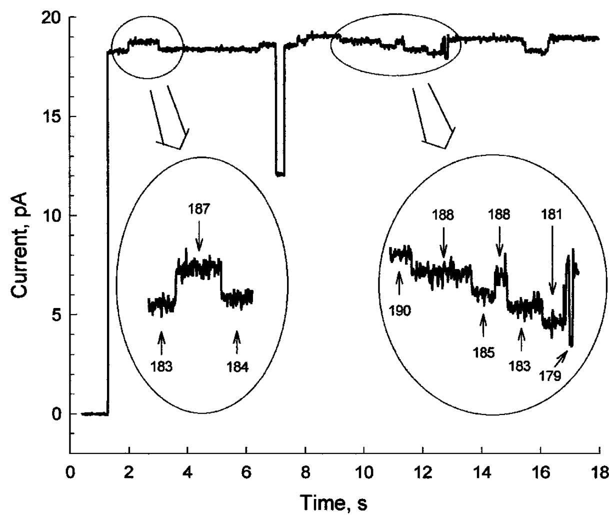

FIG. 1. Current through a single open maltoporin channel shows stepwise transitions between conductance substates. Lipid bilayer membranes of about 30 pF capacitance were formed by the monolayer opposition technique from diphytanoyl phosphatidylcholine (e.g., Ref. [17]). Membrane-bathing solution was 1 M KCl with 1 mM $CaCl_2$ and 10 mM TRIS at pH 7.4. Transmembrane voltage was 100 mV, positive from the side of protein addition. Though no temperature stabilization was used, room temperature was stable within 0.1 °C during a particular experiment. In different experiments it was in the range of 23 to 24 °C.

In the first one, the overall recording corresponding to the open state of the channel was analyzed. The result, averaged over 51 samples of 330 ms duration each and then over three different single channels, is presented in Fig. 2. The spectrum contains a $1/f$-like component, with an average slope of 1.1 at frequencies lower than 100 Hz. The magnitude of this component is close to the magnitude reported for multichannel maltoporin system. The "Hooge parameter," defined not per charge carrier as in the original work [15], but per channel [8,11], is $\alpha \approx 3 \times 10^{-5}$. This value is somewhat small but close to the range specified for the multichannel system [8].

Our second strategy was to excise the stepwise current fluctuations. This was performed with the help of the following algorithm. First, a raw record was filtered using averaging time τ. Second, a current window having half-width Δi and centered at the mean current of one of the channel substates, $\langle i_n \rangle$, was chosen. The program analyzed channel current by detecting all points of the filtered recording that were outside of the specified current window and then eliminating all parts of the initial raw recording in the $\pm\tau$ vicinity of each of such points. The remaining parts of the initial (unfiltered) recording were "glued" together after their mean values were subtracted. The resulting spectral density obtained for the following parameters: $\tau = 10$ ms, $\langle i_n \rangle = 18.4$ pA, $\Delta i = 0.17$ pA, is shown in Fig. 2. It is flat at frequencies below 100 Hz.

To check against unwanted artifacts introduced by this algorithm, especially against possible suppression of low-frequency continuous signals, we ran a special set of experiments. We admixed small-amplitude (smaller than 0.1 pA, when recalculated to the amplifier input) sine-wave signals of 25, 50, and 100 Hz to the raw channel recording *before* the signal-selecting procedure was applied. It turned out that at the parameters given above, attenuation of continuous signals by the signal-selection algorithm was negligible in comparison to the reduction of $1/f$ noise seen at low frequencies. Specifically, experiments with the 100 Hz signal demonstrated that its 3 dB attenuation was reached only when the half width of the current window was decreased to about 50–70 fA, whereas at the half width of 0.15 pA or higher the attenuation was well below the accuracy of our measurements.

Figure 2 shows that excision of stepwise changes in the channel current transformed its low-frequency $1/f$-like noise to a noise with a flat spectrum. Importantly, very close results could also be obtained using selection of transition-free fragments of 1 s and longer by visual examination of recordings. After subtracting the background, this noise component could be roughly approximated by a Lorentzian with a cutoff frequency of about 300 Hz. The intensity of its frequency-independent part is about 2.8×10^{-29} A^2/Hz. This is several times higher than the corresponding values for shot-noise, $2e\langle i \rangle = 5.8 \times 10^{-30}$ A^2/Hz (where $\langle i \rangle = 1.8 \times 10^{-11}$ A is the current

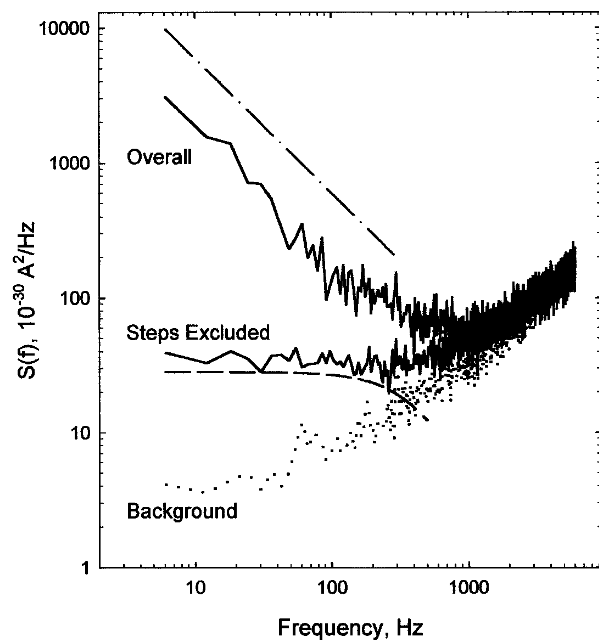

FIG. 2. Low-frequency power spectral density of the open channel noise changes from $1/f$-noise-like behavior (upper curve) to a "white" one (middle curve) when stepwise transitions in the channel current are excluded. The straight dashed-dotted line shows the $1/f$ slope. The dashed line is a Lorentzian fit to the spectrum represented by the middle curve with the background subtracted. Sampling rate was 50 kHz; the input low-pass Butterworth filter was set to 15 kHz. Other experimental conditions were as in Fig. 1.

through the single channel and e is elementary charge), or Johnson noise, $4kTg = 2.9 \times 10^{-30}$ A^2/Hz (where $g = 1.8 \times 10^{-10}$ S is the channel conductance and k and T have their usual meaning of the Boltzmann constant and absolute temperature) expected in this case. The origin of this noise is not understood at this moment. It may be related to the still unresolved open channel conformational fluctuations [16] or to the reversible recharging of ionizable residues in the channel-forming molecule [17,18]. In any case, the $1/f$ component observed in the overall recording analysis is absent here to a high accuracy. The upper estimate for the Hooge parameter obtained from the data in Fig. 2 is $\alpha \leq 3 \times 10^{-7}$.

The channel is a trimer [19]. This assertion can be readily checked by addition of 30 μm of sugar (maltohexaose) to the bulk salt solution. The corresponding current recording (Fig. 3, inset) displays three current levels. One sugar molecule binds to specific groups in the channel pore [9], completely blocking ion current through a channel monomer. Blocking of different monomers is mutually independent, so the probability to find one, two, or all three monomers blocked can be described by binomial distribution [20]. Figure 3 shows that the corresponding power spectral density is Lorentzian; the blocking of a single trimer is thus well described by a simple two-state Markovian process [21]. Addition of sugar induces vigorous switching of the channel between states differing in conductance by about 60 ps—that is, by one third of the total channel conductance. However, even in the absence of sugar, channel fluctuates between substates of a relatively close conductance. As Fig. 2 demonstrates, the dynamics of these fluctuations are far more complex.

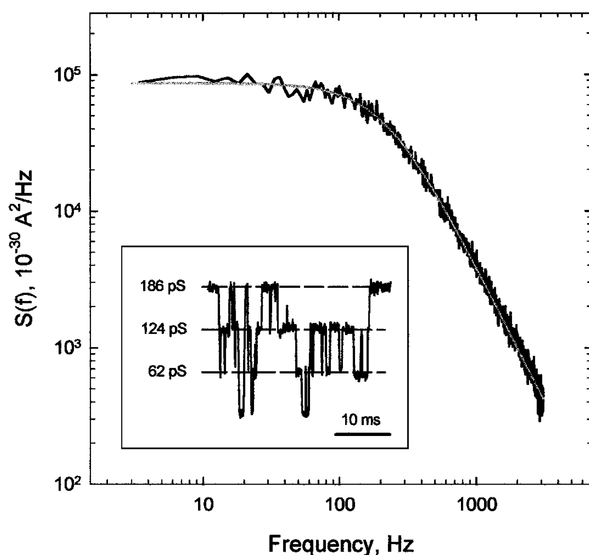

FIG. 3. Addition of sugar, maltohexaose, shows that the channel is a trimer. Sugar molecules block channel monomers independently, showing three levels of channel conductance differing by about 60 pS (inset). The spectrum of sugar-induced fluctuations is described by a single Lorentzian (smooth solid line, corner frequency 217 Hz) over 3 orders of frequency change. Maltohexaose was added symmetrically, to both sides of the cell, in a concentration of 30 μm. Other experimental conditions were as in Figs. 1 and 2.

Thus, the stepwise changes in conductance shown in Fig. 1 are responsible for $1/f$ noise generation of the open maltoporin channel. They seem to represent equilibrium conformation fluctuations between different substates of the open channel because the amplitude of $1/f$ noise component in Fig. 2 is roughly proportional to the applied voltage squared (data not shown). This kind of behavior is characteristic of the $1/f$ noise that is generated by conductance fluctuations governed by equilibrium processes [22,23]. That is, the current through the channel is only a probe of conductance fluctuations, $\delta g(t)$, that do not depend on the voltage (or current) across (through) the channel. In this case, the square of band-limited current fluctuation (whose average represents power spectral density) is $[\delta i(t)]^2 = [\delta g(t)V]^2 \propto V^2$.

As for the self-organized criticality model [13] to elucidate noise in porins [11], its serious limitations for $1/f$ noise explanation in real systems were pointed out some ten years ago [24]. It is also hard to imagine that porins, or other ion channels for that matter, are dissipative critical structures of the kind discussed by Bak and co-workers [13]. While proteins, at least to some degree, do possess self-organization property [25], they are equilibrium (though, probably, metastable [26]) objects. They are very complex as well. This complexity refers not only to their hierarchical structure with multiple time scales for motion of different domains [27,28], but, in the case of ion channels, to their intricate interactions with membrane lipids. As a consequence, it is not a big surprise that their conformational dynamics, seen as stepwise transitions between different conductance substates, can produce noise with $1/f$-like spectrum (Fig. 2). Rather, the surprise is that the process of maltohexaose interaction with the channel is described by a "pure" Lorentzian (Fig. 3). This process includes several steps such as diffusion of the sugar molecule to a binding structure inside the channel pore (the "greasy slide" and "ionic tracks" [14]), binding, random jumps along this structure, and consequent release; at the same time the spectrum suggests that the whole process is perfectly described by simple two-state Markovian kinetics.

To conclude, $1/f$ noise found in bacterial porins [11] is, in fact, related to "channel breathing" first discussed in the analysis of the open channel noise of acetylcholine receptor [16]. We show that the smaller, second-order conformational fluctuations that do not lead to the complete channel closure and, thus, cannot be resolved in multichannel experiments, are responsible for the generation of $1/f$ noise. The striking inverse dependence of $1/f$ noise intensity on channel conductance reported for a series of different porins [11] and the available 3D structural data [14,19,29] suggest that these fluctuations may be related to the dynamics of the third external loop protruding into the

channel β-barrel pore. Generally, our results demonstrate that $1/f$ noise in ion channel currents is not a fundamental property of nonequilibrium transport phenomena; rather, it reflects the complex hierarchy of equilibrium protein dynamics that modulate channel conductance.

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