

Fluctuations of Ionic Current Through Lipid Bilayers at the Onset of Peptide Attacks and Pore Formation

G. C. Fadda

Université Paris 13, UFR SMBH, 93017 Bobigny cedex and Laboratoire Léon Brillouin, CEA/CNRS UMR 12, CEA-Saclay, 91191 Gif-sur-Yvette cedex, France

D. Lairez

Laboratoire Léon Brillouin, CEA/CNRS UMR 12, CEA-Saclay, 91191 Gif-sur-Yvette cedex, France

G. Zalczer

Service de Physique de l'Etat Condensé, CEA Saclay, 91191 Gif-sur-Yvette cedex, France

(Received 11 April 2009; published 27 October 2009)

Voltage-clamp measurements on lipid bilayers at the onset of peptide attacks before pore formation are reported. With four different peptides [alamethicin, melittin, and two synthetic peptides of the leucine (L)–lysine(K) copolymers (LK series)], correlations of conductivity fluctuations slowly decay over four decades in time. This slow dynamics is interpreted as being due to fluctuations of peptide concentration at the crowded surface of the bilayer and found to be compatible with the $t^{-1/2}$ relaxation of the RSA model.

DOI: 10.1103/PhysRevLett.103.180601

PACS numbers: 87.16.dp, 05.40.–a, 68.43.De

Many small peptides are known for their ability to make holes in cell membranes. These peptides are either natural (e.g. melittin from the bee venom; alamethicin from the fungus *Trichoderma viride*) or synthetic [e.g., leucine (L)–lysine(K) copolymers (the LK series)]. They have been extensively studied, originally because of their antibiotic potential source [1] and more recently for their promising anticancer properties [2] and gene therapy applications [3]. Although they have been extensively studied, the exact mechanism of the membrane damages they produce is still debated [4]. Two mechanisms are reported depending on the peptide. In the first one, known as “detergentlike”, peptides dissolve the bilayer by forming comicelles with lipids and each departing comicelle leaves a hole in the membrane [5]. In the second mechanism, known as “channel-forming”, peptides form distinctive pores involving a given number of molecules in a “barrel-stave” (alamethicin seems to be the only peptide of this category) or “toroidal” (e.g. melittin) structure [6]. These mechanisms share a common feature: hydrophilic and hydrophobic residues of these rodlike peptides are located on opposite sides of the rod axis (amphipathy), and it is well established experimentally that, prior to pore formation, they adsorb parallel onto the membrane surface up to a high given surface density at which pores begin to be observed.

The pores can be detected by monitoring the ionic current flowing through these channels once a small potential is applied across the bilayer (“voltage-clamp” measurements). Experiments concerned with single pore have shown that such systems fluctuate between many conductivity levels which are usually interpreted as resulting either from the successive addition of one peptide in the

perimeter of the hole [7] or from fluctuations of its inner structure [8]. For these highly conductive states, in most of the cases reported in the literature, finite lifetimes are observed (see for instance [9]), that correspond to an almost exponential decay of correlations. In some cases, anomalous and slow relaxation of correlations (identified in the frequency domain as a “ $1/f$ noise”) are reported [10,11]. However, a general behavior failed to emerge.

Our purpose in this Letter is to study lower conductivity state, with a most probable conductivity level smaller than the conductivity of one pore, and which we consider as a precursor state to pore formation. As is often the case near a transition, the dynamics in this regime might provide clues to the trigger of pore opening. Indeed the time autocorrelation function of ionic current exhibited a slow power law decrease, from which no average lifetime can be computed. This is reminiscent of many-body problems near a jamming transition [12] as the one occurring in random sequential adsorption (RSA) [13], rather than single particles dynamics. We show that four different peptides exhibited this generic feature.

Samples characteristics and preparation.—Experiments were done with a 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) bilayer (Avanti Polar Lipids). A stock solution of 10 mg/ml is prepared in decane. The bilayer is formed according to standard techniques [14] on the pinhole (120 μm diameter) of a perforated vessel. The pinhole is coated with a droplet of DPhPC solution, decane is evaporated next during 30–40 min at room temperature. The vessel is then put between two chambers filled with 900 μl of a 1M KCl solution. A pipette with the lipid solution is then used to brush the coated aperture in order to form the bilayer which is detected measuring the current

between two Ag-Cl electrodes. From intensity and current phase measured with a 10 Hz sinus voltage-clamp, the capacitance of the membrane was found to lie between 40 and 60 pF depending on the preparation. Two cationic peptides of the LK series were used. K(LK)₇ (denoted LK15 β) folds to an antiparallel β -sheet secondary structure when it interacts with lipid membrane [15]; K(LLKL)₄LK (denoted LK19 α) folds in an amphiphilic α -helix secondary structure [16]. LK15 β , LK19 α (Neosystem-France, more than 95% pure) and Melittin (Sigma-Aldrich, more than 97% pure) were dissolved in water at an initial concentration of 35 μ M. Alamethicin (Sigma-Aldrich, more than 90% pure) was dissolved in ethanol and diluted with water at 77 μ M. After formation of the bilayer, peptide solution is introduced in one (or both) compartment.

Data acquisition and treatment.—Voltage-clamp (membrane voltage: 100 mV) and ionic current amplification were ensured by an Axopatch 200B with a 10 kHz low-pass analog filter setting. The amplified current was digitized with a 16 bits analog-digital converter (Iotech Wavebook) at 200 kHz sampling rate and averaged over 20 samples. Power spectrum density was averaged following the periodogram method [17] over 128 time segments, then inverse Fourier transform gives the time dependent autocorrelation function of the current intensity. All measurements were performed at room temperature. Prior to any peptide addition in the solution, the lipid bilayer is an insulator with a typical capacitance of about 40 pF. The zero in average conductivity is a gaussian noise with standard deviation of 10 pS.

Results.—Introducing a sufficiently large amount of peptides into one of the two compartments separated by the bilayer produces a huge increase of the conductivity. Figure 1, left shows a fraction of the signal so obtained with alamethicin. An accurate analysis of the data shows that the membrane conductivity varies with well-defined discrete and equidistant levels corresponding to peaks on the average distribution function $p(G)$ (Fig. 1, right). The conductivity increment is found to be of the order of 1000 pS (Fig. 2), in agreement with the conductivity of

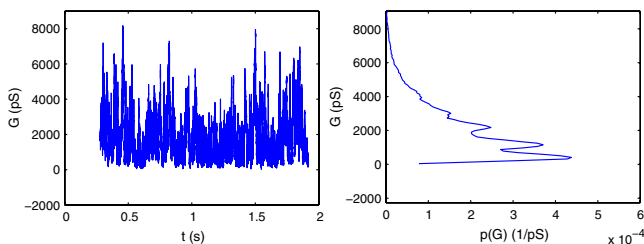


FIG. 1 (color online). High level of conductivity corresponding to multiple concomitant pores with alamethicin. Left: details of the recorded signal, ionic conductivity G vs time t . Right: distribution function, $p(G)$, of the conductivity averaged over 25 s.

one single pore having the most stable conformation made of six peptide molecules [18].

In this Letter, we focus on conductivity fluctuations observed at low conductivity level (<1000 pS). This is achieved by a progressive increase of peptide concentration up to the onset of membrane attacks. This occurs at concentration between 0.1 and 1 μ M, depending on the peptide. Most of the experiments were performed with peptide addition to one side of the bilayer, however it was checked that peptide addition to both sides does not modify our observations. Figures 3–6 show typical results for the four peptides we used. On these figures, a sample of the conductivity G is zoomed in for a close up of fluctuations over $T \approx 1.6$ s (top-left) and the distribution function averaged over the total record duration is plotted (top-right). The most probable value for the current intensity (peak for $p(G)$) is always at a level smaller than the one expected for a single pore. The vicinity of the zero conductance level implies that these distribution functions are highly asymmetric, contrary to the fluctuations observed around the conductivity level of a perennial pore. On the bottom of Figs. 3–6, the time dependent autocorrelation of the conductivity, $g(\tau) = \langle G(t)G(t + \tau) \rangle - \langle G \rangle^2$, is plotted. In the four cases, although the details of fluctuations differ their autocorrelation functions are quite similar and reveal an unexpected slow relaxation that extends over several decades in time. This decrease is too slow to allow for computing a finite first moment of $g(\tau)$ providing a characteristic time.

Discussion.—Let us first remember that at room temperature, a DPhPC lipid bilayer is liquid and displays dynamics with characteristic time scales in the nanosecond range (e.g., see [19]). In the ideal solution limit, the insertion of a given peptide inside a bilayer is controlled by the ratio of the thermal energy to an energy barrier. The probability of this occurrence is constant in time, which

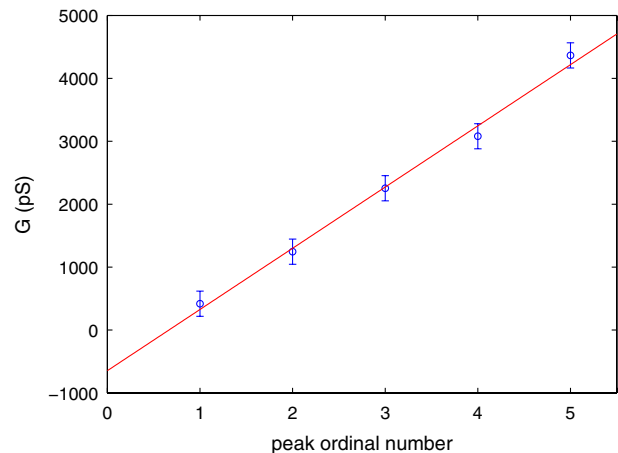


FIG. 2 (color online). High level of conductivity corresponding to multiple concomitant pores with alamethicin: discrete conductivity levels vs ordinal number. The straight line has a slope equal to (975 ± 30) pS.

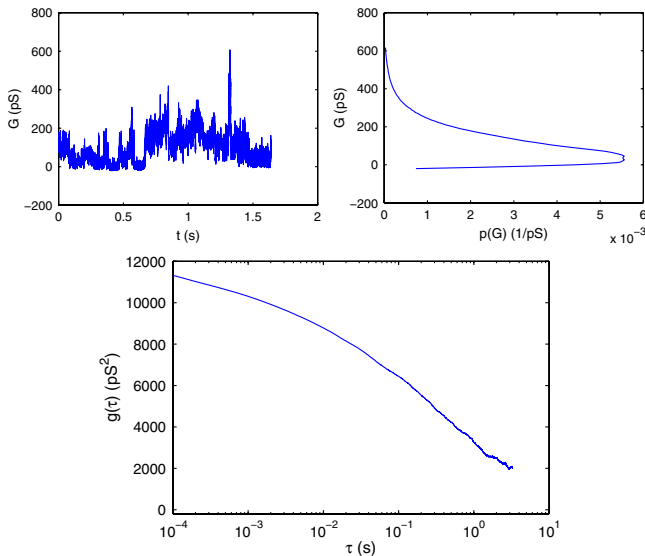


FIG. 3 (color online). Low level of conductivity obtained with alamethicin. Top-left: details of the recorded signal, conductivity G vs time t . Top-right: distribution function, $p(G)$, of the conductivity averaged over 800 s. Bottom-right: autocorrelation function of conductivity fluctuations $g(\tau) = \langle G(t)G(t + \tau) \rangle - \langle G \rangle^2$ averaged over the same duration.

leads to an exponential time behavior with a well-defined characteristic time. Such exponential behavior is still expected even if we consider the case of the addition of a given peptide into a preexisting pore. Depending on this preexisting pore, this latter case might lead to a time distribution that can hardly extend over several orders of magnitude. Actually, slow dynamics (slower than an exponential) is usually met in collective motions that slow down near a fluid to solid transition known as “jamming

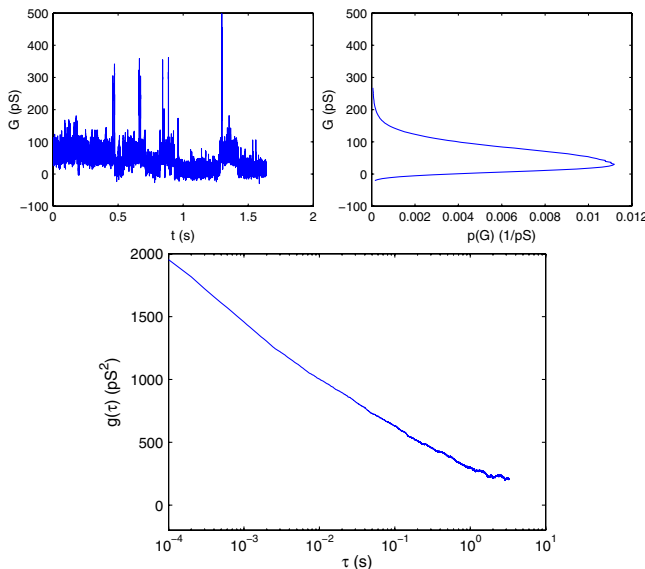


FIG. 4 (color online). Low level of conductivity obtained with Melittin. Same plots as in Fig. 3.

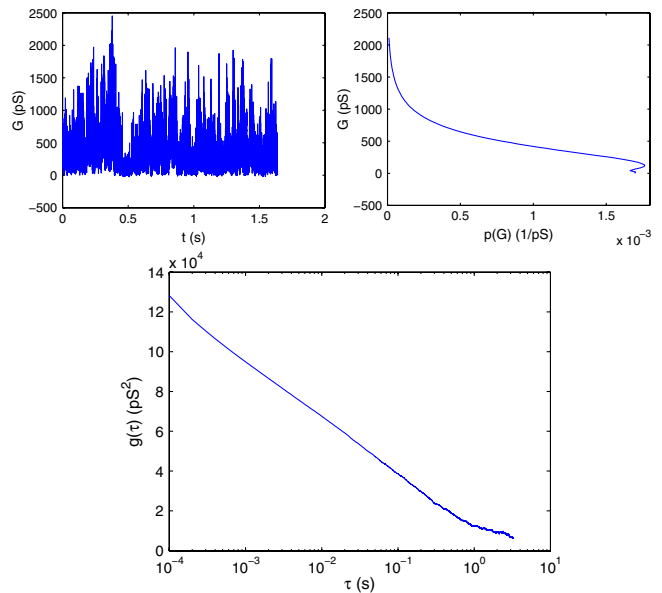


FIG. 5 (color online). Low level of conductivity obtained with LK15 β . Same plots as in Fig. 3.

transition” [12]: nearly log-decay relaxation of fluctuations are reported for supercooled liquids [20]; granular media [21]; gelation of attractive colloidal particles [22] or copolymer micelles [23]. “Random sequential adsorption” (RSA) is an early and simple model that initially aims to account for slow dynamics of jammed systems [13]. It considers the filling process of a surface with random deposition of particles allowed (or not, depending on the model refinement) to diffuse at the surface. This model has been already invoked for the understanding of protein adsorption kinetics (see for instance [24] and for a recent

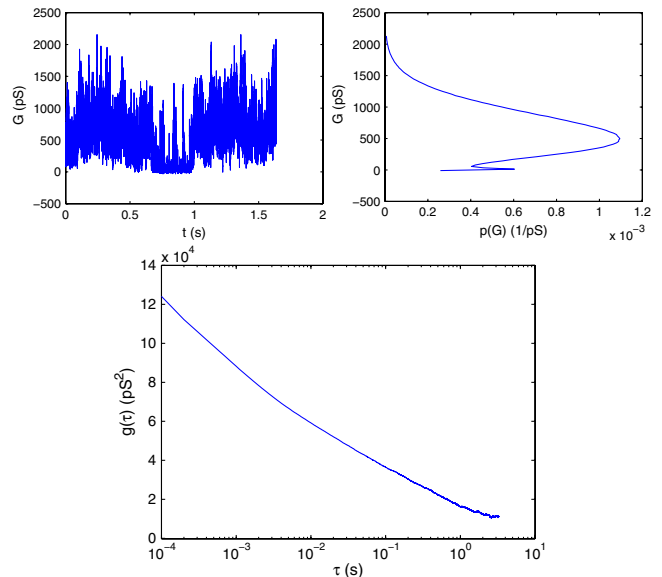


FIG. 6 (color online). Low level of conductivity obtained with LK19 α . Same plots as in Fig. 3.

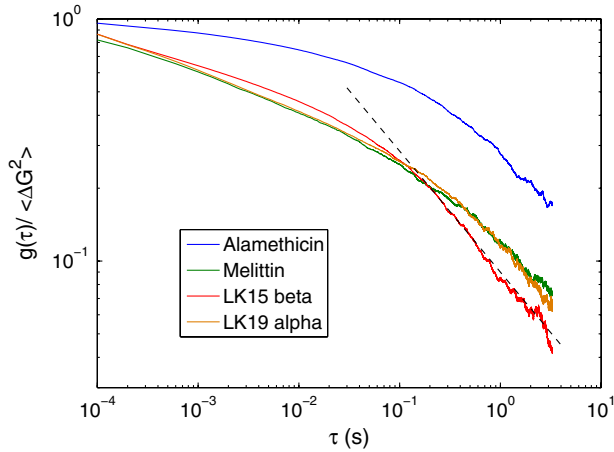


FIG. 7 (color online). Normalized autocorrelation function of conductivity, $g(\tau)/\langle\Delta G^2\rangle$, vs τ . Dashed line is a guide for the eyes corresponding to $\tau^{-1/2}$ expected from RSA model [Eq. (1)].

review [25]). It is particularly realistic for the random adsorption of peptides onto a lipid bilayer that is known as the first stage of pore formation. The coverage density, $\rho(t)$, increases with time up to the equilibrium density $\rho(\infty)$ (i.e., the closest-packing density if diffusion is allowed or the jamming limit density if not). The density approaches the equilibrium as [26,27]

$$\rho(\infty) - \rho(t) \propto t^{-1/2}. \quad (1)$$

Fluctuation-dissipation theorem let us expect the same behavior for the density autocorrelation function.

Then, the key point of our interpretation is the following: Peptides are adsorbed parallel to the surface of the bilayer to such a large amount that they interact strongly with one another. The formation of a pore implies that some adsorbed peptides reorient perpendicular and insert inside the bilayer [6]. Thermodynamic equilibrium supposes a continual exchange of peptides between these two populations. Assuming that the latter is directly linked to the ionic current intensity, the time evolution of this current is therefore directly related to the fluctuations of peptide concentration at the crowded surface. The dynamics of these fluctuations is expected to obey RSA model.

In Fig. 7, the normalized autocorrelation function of fluctuations of conductivity, $g(\tau)/\langle\Delta G^2\rangle$ is plotted in a log-log scale and compared with Eq. (1). In the four cases, the shape of the autocorrelation function at long time is quite compatible with a $t^{-1/2}$ decay. Note that for melittin, LK15 β and LK19 α , the curves are almost superimposable. The quite peculiar “barrel-stave” mechanism for the formation of alamethicin pores could be responsible for a distinctive short time dynamics responsible for a shift of the correlation function.

These results give a new insight of peptides induced pores that could be viewed as quenched or jammed structure. In particular, it should be valuable to reconsider

interpretations of the “ $1/f$ noise” reported for a long time on stable peptide-pores obtained *in vitro* [10] or in a crowded *in vivo* environment [28].

Thanks to L. Auvray, J. Mathé, and J. Pelta for helping us to obtain our first voltage-clamp signals.

- [1] R. E. W. Hancock and R. Lehrer, *Trends Biotechnol.* **16**, 82 (1998).
- [2] D. W. Hoskin and A. Ramamoorthy, *Biochim. Biophys. Acta, Biomembranes* **1778**, 357 (2008).
- [3] M. Martin and K. Rice, *AAPS J.* **9**, E18 (2007).
- [4] H. Sato and J. B. Feix, *Biochim. Biophys. Acta, Biomembranes* **1758**, 1245 (2006).
- [5] E. Gazit, A. Boman, H. G. Boman, and Y. Shai, *Biochemistry* **34**, 11479 (1995).
- [6] M.-T. Lee, W.-C. Hung, F.-Y. Chen, and H. W. Huang, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 5087 (2008).
- [7] G. Boheim, *J. Membr. Biol.* **19**, 277 (1974).
- [8] H. N. L. Vogel, R. Rigler, S. Meder, G. Boheim, H.-H. Beck, W. Kurth, and G. Jung, *Eur. J. Biochem.* **212**, 305 (1993).
- [9] H.-A. Kolb and G. Boheim, *J. Membr. Bio.* **38**, 151 (1978).
- [10] R. Sauv e and E. Bamberg, *J. Membr. Bio.* **43**, 317 (1978).
- [11] F. Sigworth, D. Urry, and K. Prasad, *Biophys. J.* **52**, 1055 (1987).
- [12] A. J. Liu and S. R. Nagel, *Nature (London)* **396**, 21 (1998).
- [13] J. W. Evans, *Rev. Mod. Phys.* **65**, 1281 (1993).
- [14] P. Mueller, D. Rudin, R. Tien, and W. Wescott, *J. Phys. Chem.* **67**, 534 (1963).
- [15] S. Castano, B. Desbat, and J. Dufourcq, *Biochim. Biophys. Acta, Biomembranes* **1463**, 65 (2000).
- [16] S. Castano, B. Desbat, M. Laguerre, and J. Dufourcq, *Biochim. Biophys. Acta, Biomembranes* **1416**, 176 (1999).
- [17] A. Oppenheim and R. Schaffer, *Digital Signal Processing* (Prentice-Hall Inc., Englewood Cliffs, NJ, 1975).
- [18] D. P. Tieleman, B. Hess, and M. S. P. Sansom, *Biophys. J.* **83**, 2393 (2002).
- [19] W. Shinoda, M. Mikami, T. Baba, and M. Hato, *Chem. Phys. Lett.* **390**, 35 (2004).
- [20] H. Cang, V. N. Novikov, and M. D. Fayer, *Phys. Rev. Lett.* **90**, 197401 (2003).
- [21] J. Bruji c, P. Wang, C. Song, D. L. Johnson, O. Sindt, and H. A. Makse, *Phys. Rev. Lett.* **95**, 128001 (2005).
- [22] V. Trappe, V. Prasad, L. Cipelletti, P. N. Segre, and D. A. Weitz, *Nature (London)* **411**, 772 (2001).
- [23] J. Grandjean and A. Mourchid, *Europhys. Lett.* **65**, 712 (2004).
- [24] O. Michielin, G. Verg eres, and J. J. Ramsden, *J. Am. Chem. Soc.* **121**, 6523 (1999).
- [25] Z. Adamczyk, M. Nattich, and J. Barbasz, *Adv. Colloid Interface Sci.* **147–148**, 2 (2009).
- [26] R. H. Swendsen, *Phys. Rev. A* **24**, 504 (1981).
- [27] E. Eisenberg and A. Baram, *Europhys. Lett.* **44**, 168 (1998).
- [28] H. Derksen and A. A. Verveen, *Science* **151**, 1388 (1966).