Arguments in favor of an aggregational model of the gramicidin channel: a reply

Dear Sir:

Studies on the behavior of ion channels formed by gramicidin A in planar (black) lipid membranes are frequently explained on the basis of a dimer model originally suggested by Urry (1971). The fluctuations accompanying the equilibrium between two helical monomers and a head-to-head dimer indeed allow a straightforward interpretation of single-channel data. Kinetic studies including temperature-jump and voltage-jump experiments were, however, at variance with this simple model (Stark et al., 1986). The corresponding data could be understood in the frame of an aggregational (micellar) model. It is based on lateral aggregation of gramicidin-dimers and assumes that isolated dimers are nonconducting, but may be converted to the conducting state on lateral association of gramicidin dimers. The possibility of dimer–dimer interactions specifically induced by tryptophan–tryptophan contacts was first discussed by Urry (1972) and was subsequently confirmed experimentally by Cavatorta et al. (1982), Henze et al. (1982), and Spisni et al. (1983). It was also suggested that the tendency of gramicidin to self-associate may strongly influence the structure of lipid membranes, i.e., may change the temperature of phase transitions or may trigger the transition of a micellar structure into that of a bilayer structure (for a review, see Killian and De Kruijff, 1986).

The question whether isolated gramicidin dimers form open ion channels was again addressed in a recent article by Cifu et al. (1991). The authors performed single channel studies with hybrid channels observed in the presence of different gramicidin analogues, and come to the conclusion that "the elementary conductance unit is a (isolated) dimer." An alternative explanation was suggested for the kinetic studies by Stark et al. (1986).

The arguments put forward by Cifu et al. (1991) consider, however, only part of the experimental evidence in favor of the aggregational model. In addition, an important part of their objections is based on a theoretical argument that (at least according to the author's opinion) is insufficient to exclude self-aggregation.

The aggregational model is based on the following experimental findings (Stark et al., 1986, Strässle et al., 1989).

(*a*) The relaxation of the electric current induced by a voltage-jump shows at least two (presumably three) different relaxation times. This is indicative of a process of channel activation that includes three or more different system states in contrast to the simple two-state monomer-dimer model.

(*b*) The relaxation behavior was found to be largely identical for normal gramicidin A and for chemically dimerized malonyl-bis-desformylgramicidin. Therefore, system states comprising aggregates of dimers must be assumed to explain the relaxation behavior.

(*c*) The formation rate of open gramicidin channels is strongly reduced if the membrane and its aqueous environment are irradiated by ionizing radiation. This was shown to be the consequence of an interaction of free radicals OH· and HO_2· (produced by water radiolysis) at one of the tryptophan residues of the channel. The replacement of the tryptophan residues by other aromatic residues leads to a reduction by several orders of magnitude of the radiation sensitivity of the gramicidin channels. The tryptophan residues (according to Urry's model of the dimer structure) are located at the COOH-terminal end of gramicidin A in close contact with the aqueous phase. The coupling of two monomers to a dimer, however, occurs at the NH_2-terminal end that is not affected by irradiation. Therefore, the reduced rate of channel formation of irradiated gramicidin A can hardly be attributed to a reduced rate of dimer formation. On the other hand, the lateral aggregation of gramicidin-dimers, favored by tryptophan–tryptophan contacts, should react very sensitively to a chemical modification of the latter.

(*d*)$_4$ The importance of the tryptophan residues for the formation of open ion channels may also be concluded from experiments with naphthylanalogues of gramicidin A, where part of the tryptophan residues are replaced by naphthylalanines (not published in detail). The concentration of these analogues in the membrane forming solution (or in the aqueous phase) must be increased by one to two orders of magnitude (as compared with normal gramicidin A) to obtain the same membrane conductance. This was found to be primarily the result of a strongly reduced formation rate of open channels. Following the same line of argumentation as above, the dimerization process should not be affected by the chemical modification at the COOH-terminal end. On the other hand, dimer aggregation (due to the lack of favoring tryptophan interactions) may be expected to be strongly reduced.

The paper by Cifu et al. (1991) only deals with the first and second argument, namely with a relaxation behavior, which is in clear disagreement with the simple mono-merdimer model. Cifu et al. (1991) suggest a modification of this model by introducing transitions between two different conformations of monomers in the membrane, with only one conformation being able to form dimers. Such a model (as any three-state model) predicts two different relaxation times in a voltage-jump experiment, and has also been considered by us as one of several alternatives to the aggregational model to explain the general shape of the relaxation curves (unpublished). The model is, however, at variance with our second argument, namely the largely identical relaxation behavior of gramicidin in the monomeric and dimeric form. Cifu et al. (1991) resolve the discrepancy by assuming a contamination of the sample of chemically dimerized gramicidin A by monomer gramicidin A. It is difficult to argue against such an argument, because the sample was not prepared by ourselves. Bamberg and Janko (1977) used LH 20 Sephadex chromatography, UV-, IR-, and NMR-spectroscopy, as well as a determination of the molecular weight (by use of an analytical ultracentrifuge) to demonstrate the purity of the sample. There is (contrary to the statement by Cifu et al. [1991]) no conflict between the single channel data and the relaxation data of malonyl-bis-desformyl-gramicidin. It is not permissible to average several classes of single channel events and to compare the mean channel life

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time obtained with one of the individual relaxation times. Our single channel data showed at least three different classes of events (Stark et al. 1986; see also noise analysis by Sauvé and Szabo [1985]). One class of events compares well with the fast relaxation time. The reproach of contamination through monomer gramicidin A by Cifu et al. (1991) would have been more convincing if the authors had prepared a new sample of malonyl-bis-desformylgramicidin devoid of contamination (as shown by a different relaxation behavior).

Cifu et al. (1991) try to exclude dimer aggregation by performing studies on hybrid channels between different gramicidin analogues, a technique introduced by Veatch and Stryer (1977) and by Apell et al. (1977). Only two different types of hybrid channels were detected, as was also reported in the previous studies. The experiments by Cifu et al. (1991) certainly represent an improvement with respect to the separation of different conductance peaks. Their study (according to the author's view) is nevertheless insufficient to disprove aggregation of dimers as a prerequisite of channel opening. They try to show that there is no association between conducting dimers and nonconducting monomers by performing single channel hybrid experiments with normal gramicidin A (gA) and with the negatively charged analogue *O*-pyromellitylgramicidin (OPgA). They argue that by association of OPgA to a conducting gA-dimer, the conductance of the latter should be increased. The argument is based on an enhanced cation concentration at the entrance of the channel, which is generated through an electrostatic effect of adsorbed OPgA. Since an effect of OPgA on the gA-conductance was not observed, the absence of aggregation was concluded.

The argument relies heavily on application of electrostatic theories and on the geometric distance of the charges within an aggregate. The latter can only be estimated rather crudely. While there is usually good agreement between theory and experiment at homogeneously charged surfaces, the effect of local charges on its immediate environment is difficult to predict (if considered on a quantitative basis). This is also apparent from the article of Cifu et al. (1991). On the one hand they find "little detectable electrostatic repulsion between the charged groups at the entrance of symmetrical OPgA channels" (i.e., between the two monomers in an isolated dimer), in clear contradiction to their own estimates of electrostatic forces. Nevertheless, they use the same argument to estimate the electrostatic effect on the single channel conductance over the distance of two adjacent dimers and to deduce the absence of dimer aggregation.

New evidence for dimer aggregation (based on single channel studies of covalently linked [with 3,3′-azobis (benzeneacetic acid)] gramicidin monomers) was recently provided by Stankovic et al. (1991). For steric reasons this special dimer (in the isolated state) cannot provide a free pathway for the movement of ions if the linker is in the *trans*-form. Nevertheless, single channel fluctuations were observed and were consequently interpreted in the framework of intermolecular gramicidin aggregates. Aggregation, however, was considered only for the analogue and not for normal gramicidin A by Stankovic et al. (1991).

The author of this letter is convinced that there is overwhelming experimental evidence for a dimer (whether isolated or aggregated) to represent the elementary conductance unit of a (normal) gramicidin A channel. There is certainly less evidence that the stabilization of the dimers, in order to form open ion channels, requires lateral aggregation. A number of experimental findings, however, are difficult to understand without such an assumption. The studies available at present (according to the author's view) are insufficient to provide a final answer to this controversial question.

REFERENCES

Apell, H.-J., E. Bamberg, H. Alpes, and P. Läuger. 1977. Formation of ion channels by a negatively charged analog of gramicidin A. *J. Membr. Biol.* 31:171–188.

Bamberg, E., and K. Janko. 1977. The action of a carbonsuboxide dimerized gramicidin A on lipid bilayer membranes. *Biochim. Biophys. Acta.* 465:486–499.

Cavatorta, P., A. Spisni, E. Casali, L. Lindner, L. Masotti, and D. W. Urry. 1982. Intermolecular interactions of gramicidin A transmembrane channels incorporated into lysophosphatidylcholine lipid systems. *Biochim. Biophys. Acta.* 689:113–120.

Cifu, A. S., R. E. Koeppe II, and O. S. Andersen. 1991. On the supramolecular organization of gramicidin channels. The elementary conducting unit is a dimer. *Biophys. J.* 61:189–203.

Henze, R., E. Neher, T. L. Trapane, and D. W. Urry. 1982. Dielectric relaxation studies of ionic processes in lysolecithin-packaged gramicidin channels. *J. Membr. Biol.* 64:233–239.

Killian, J. A., and B. De Kruijff. 1986. The influence of proteins and peptides on the phase properties of lipids. *Chem. Phys. Lipids.* 40:259–284.

Sauvé, R., and G. Szabo. 1985. Interpretation of 1/f fluctuations in ion conducting membranes. *J. Theor. Biol.* 113:501–516.

Spisni, A., I. Pasquali-Ronchetti, E. Casali, L. Lindner, P. Cavatorta, L. Masotti, and D. W. Urry. 1983. Supramolecular organization of lysophosphatidylcholine-packaged gramicidin A′. *Biophys. Biochim. Acta.* 732:58–68.

Stankovic, C. J., S. H. Heinemann, and S. L. Schreiber. 1991. Photo-modulated ion channels based on covalently linked gramicidins. *Biophys. Biochim. Acta.* 1061:163–170.

Stark, G., M. Strässle, and Z. Takácz. 1986. Temperature-jump and voltage-jump experiments at planar lipid membranes support an aggregational (micellar) model of the gramicidin A ion channel. *J. Membr. Biol.* 89:23–37.

Urry, D. W. 1971. The gramicidin A transmembrane channel: a proposed $\pi_{(L,D)}$ helix. *Proc. Natl. Acad. Sci. USA.* 68:672–676.

Urry, D. W. 1972. A molecular theory of ion conducting channels: a field dependent transition between conducting and nonconducting conformations. *Proc. Natl. Acad. Sci. USA.* 69:1610–1614.

Veatch, W. R., and L. Stryer. 1977. The dimeric nature of the gramicidin A transmembrane channel: conductance and fluorescence energy transfer studies of hybrid channels. *J. Mol. Biol.* 113:89–102.

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