J. Membrane Biol. 50, 257–270 (1979)

Formation of Ionic Channels in Black Lipid Membranes by Succinic Derivatives of Gramicidin A

E. Bamberg, H. Alpes, H.-J. Apell, R. Bradley*, B. Härter, M.-J. Quelle, and D.W. Urry**

Fachbereich Biologie, Universität Konstanz, D-7750 Konstanz, West Germany

Received 16 May 1979; revised 29 June 1979

Summary. Different succinyl derivatives of Gramicidin A were synthesized and their activity was investigated with different methods on lipid bilayer membranes. The succinyl derivatives of Gramicidin A can be classified as three different types, the O-succinyl derivative, the N-succinyl derivative and the N-O-succinyl derivative of Gramicidin A. An O-pyromellityl-N-succinyl gramicidin was synthesized which can be attributed to the latter class. It was found that O-succinyl gramicidin behaves like the unmodified Gramicidin A despite a charge effect on single-channel conductance, arising from the negative charge of the succinic residue at the mouth of the channel. The activity of N-succinyl and N-O-succinyl gramicidin and of O-pyromellityl-N-succinyl-gramicidin depends strongly on the pH of the electrolyte solution. It is demonstrated that at low pH ($\leqq 5$) the N-succinyl derivatives show high activity, whereas at high pH ($\geqq 7$) the activity is sharply reduced or disappears totally. From these experiments it can be concluded that, for the formation of a dimeric gramicidin channel, the hydrogen of the formyl group can be replaced by a protonated carboxylic group of a succinic residue.

Further results, obtained by measurement of the single-channel conductance and of the reaction rate constants for the channel formation, are discussed in terms of the structural basis of the single stranded model for the gramicidin channel. On this basis the double stranded helix can be excluded and an interesting head-to-head single stranded $\beta(\pi_{L,D})$ helical channel is described which contains carboxyl groups at the head-to-head junction.

Gramicidin A, a linear hydrophobic pentadecapeptide isolated from *Bacillus brevis*, forms cationic selective channels in lipid bilayer membranes as well as in natural membranes. This fact was employed in recent years in many studies using Gramicidin A as a model compound with which to investigate cationic transport by a channel mechanism crossing a hydrophobic barrier (Hladky & Haydon, 1972). For a recent survey of the literature, *see* Bamberg *et al.* (1977*a*). There exist so far two

* *Permanent address:* Neuroscience Program, Laboratory of Molecular Biophysics, University of Alabama Medical Center, Birmingham, Ala. 35294.

** *Permanent address:* Laboratory of Molecular Biophysics, University of Alabama Medical Center, Birmingham, Ala. 35294.

0022-2631/79/0050-0257 $02.80

distinct proposals for the structure of the gramicidin channel. Urry proposed a single stranded dimeric channel, consisting of a head-to-head associated $\beta(\pi_{L,D})$-helix (Urry *et al.*, 1971; Urry, 1971, 1972), whereas the alternative structure consists of a double stranded β-helix (Veatch, Fossel & Blout, 1974). Recent studies with chemically modified analogs of Gramicidin A (Bamberg & Janko, 1977; Apell *et al.*, 1977; Bamberg *et al.*, 1977*b*; Sauvé & Bamberg, 1978) gave strong evidence for the single stranded helix proposed by Urry.

Bradley *et al.* (1978) tested the activity of N-succinyl gramicidin and of an O-N-succinyl gramicidin on lipid membranes. With the obtained results they concluded an "end to end" association, they discussed the activity of the N-succinyl derivatives in terms of the possibilities of tail-to-tail, head-to-head and ester-acid head-to-head associations and they looked to future pH analysis of channel formation to determine "whether only head to head channels can form or whether other combinations of end to end channel formation can occur under some circumstances...". In the present paper, experiments with several gramicidin analogs and with emphasis on pH are described, which give clear insight into the structure of the channel.

Materials and Methods

For the synthesis of the four different gramicidin derivatives (Fig. 1*a*), commercial gramicidin was used. This product contains approximately 72% gramicidin A, 9% gramicidin B, and 19% gramicidin C.

O-succinyl gramicidin was synthesized in the same way as described previously for the synthesis of O-pyromellityl gramicidin (Apell *et al.*, 1977).

N-succinyl gramicidin was obtained from desformylated gramicidin and the succinic anhydride in the following way. 15 ml of a solution of 2×10^{-4} M desformyl gramicidin were stirred in dried pyridinium (24 hr at 40 °C) together with 2×10^{-2} M succinic anhydride. The obtained product was dried and redissolved in methanol and precipitated with H_2O; the product was again dissolved in methanol and purified by thin layer chromatography. The final product is the O-N-succinyl gramicidin.

Saponification with NaOH of the O-N-succinyl gramicidin yielded N-succinyl gramicidin. The infrared spectra showed for the O-N-succinyl gramicidin and the O-succinyl gramicidin the ester band on the ethanolamine end of the molecule, whereas in the case of N-succinyl gramicidin the ester band was absent.

N-succinyl-O-pyromellityl gramicidin was synthesized from N-succinyl gramicidin and the anhydride of the pyromellityl acid. The procedure was the same as for the previously described synthesis of O-pyromellityl gramicidin (Apell *et al.*, 1977).

The UV spectra of all gramicidin derivatives showed that the tryptophanes were not damaged by the modification of the molecule. Dioleoyl lecithin and diphytanoyl lecithin (PC 16:4CH_3) were synthesized by K. Janko (Janko & Benz, 1977). Monoolein was obtained from Nucheck Preparation, Elysian, Minnesota, and checked for purity with thin layer

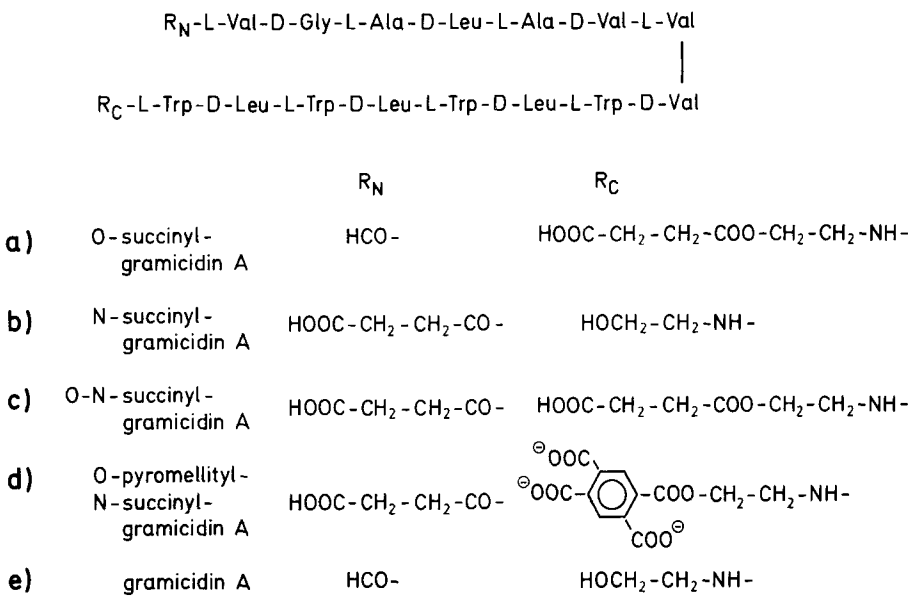

Fig. 1. Structure of the several gramicidin A derivatives

chromatography. *n*-Decane and *n*-hexadecane were obtained from Merck, Darmstadt, as standards for gas chromatography. All other reagents were analytical grade. Black membranes were formed in the usual way (Läuger *et al.*, 1967) in a thermostated Teflon cell filled with an aqueous electrolyte solution. The lipids were dissolved in *n*-decane or *n*-hexadecane (1–3% wt/vol). Different types of teflon cells were used with different holes in the septum. The membrane area was varied between 8×10^{-3} cm^2 and 8×10^{-2} cm^2 for macroscopic conductance measurements. For single-channel experiments the area of the membrane was about 3×10^{-4} cm^2. The different gramicidin derivatives were added to the electrolyte from methanolic stock solutions. Relaxation experiments and single-channel experiments were performed as described previously (Bamberg & Läuger, 1973, 1974).

Results and Discussion

pH Effects on the Macroscopic Conductance and the Single-Channel Conductance

a) O-Succinyl Gramicidin

Table 1 shows the single-channel conductance of O-succinylgramicidin at different pH and different concentrations of the transported cation. At high pH, the single-channel conductance deviates at low concentration remarkably from that of the unmodified gramicidin. At low pH $\leqq$ 4,

Table 1. Single channel conductance Λ of the different gramicidin A derivatives at different ionic concentration and two different pH[a]

N-succinyl gramicidin			O-succinyl gramicidin[b]		O-pyromellityl-N-succinyl gramicidin	
C/M	Λ/pS pH=4	Λ/pS pH=8	Λ/pS pH=4	Λ/pS pH=8	Λ/pS pH=5.0	Λ/pS pH=8
0.001	–	–	–	–	2.5	2.3
0.01	–	–	–	–	5.2	5.2
0.05	3	3	–	–	8.0	8.0
0.1	6	6	15.5 (17.7)	26	8.2	8.2
0.2	7.5	7.5	–	–	9.0	–
0.5	10.8	10.8	44 (49)	59	11.0	11.0
1.0	12.5	12.5	80 (84)	80	13.0	13.0

[a] $V=100$ mV; Electrolyte, CsCl; $T=25$ °C; lipid monooleine/*n*-hexadecane, 1% wt/vol.
[b] The single-channel conductance for unmodified gramicidin A is given in brackets and is consistent with the data given by Hladky and Haydon (1972).

however, the single-channel conductance is very similar over the whole measured range of ion concentration to that of unmodified gramicidin A. The conclusion of this experiment is that the O-succinyl group must be located at the mouth of the channel, where this group can be titrated by lowering the pH in the external medium. Similar experiments were carried out with O-pyromellityl gramicidin (Apell *et al.*, 1977). But with the O-pyromellityl derivative no detectable titration effects could be observed.

b) N-Succinyl Gramicidin

1. Multichannel experiments; pH dependence of the macroscopic membrane conductance. In a recent paper (Bradley *et al.*, 1978), it was shown that N-succinyl gramicidin is active in forming ionic channels in monooleine *n*-decane membranes. The single-channel conductance, however, is smaller and the mean lifetime is remarkably reduced compared to unmodified gramicidin A.

Figure 2 shows the change of the conductance of a lipid bilayer after addition of a certain amount of N-succinyl gramicidin to the bath solution of the membrane.

At high pH, where the carboxylic group is deprotonated, the conductance stays low. Lowering the pH to values of the range of 4, where

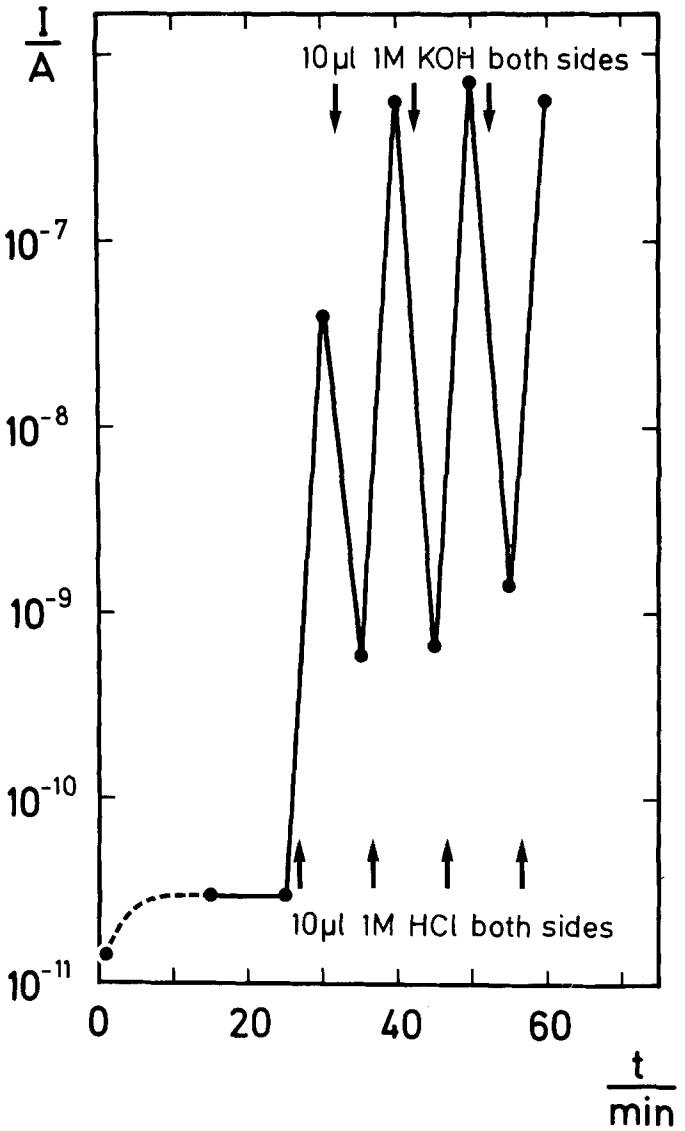

Fig. 2. Dependence of the membrane current on the pH in presence of N-succinyl gramicidin. 1 M CsCl; $T=25°$; applied voltage, 15 mV; lipid PC 16:4 CH_3 1% in *n*-decane (wt/vol). N-succinyl gramicidin was added at approximately 10^{-9} M, and pH began at 8 and oscillated approximately between 4 and 6

the carboxylic group is protonated, an increase of several orders of magnitude of the membrane conductance can be observed. Increasing the pH leads again to a dramatic reduction of the membrane conductance.

It should be noted that the experiment was carried out with the same membrane by changing the pH of the electrolyte solution.

2. Single-channel experiments. For different reasons, it was of interest to analyze the single-channel conductance of N-succinyl gramicidin. Such an investigation should clear up how the macroscopic conductance depends so strongly on the pH.

Table 1 contains the values of the single-channel conductance of N-succinyl gramicidin measured between pH 4 and 8 at different electrolyte concentrations. On increasing the pH no change of the single-channel behavior occurs, but the concentration of the peptide must be increased dramatically to get single channels. This result provides an explanation of the strong dependence of the macroscopic conductance on the pH of the electrolyte. The intrinsic parameters of the channels are invariant with changes in the pH, whereas the number of channels incorporated in the membrane is strongly decreased by increasing the pH. It is also important for the discussion of the structure of the gramicidin channel that the N-succinyl derivative does not show any charge effects on single-channel conductance at low concentration of the electrolyte in the deprotonated state of the molecule, as was found with O-succinyl gramicidin.

c) O-pyromellityl-N-succinyl Gramicidin

Bradley *et al.* (1978) described in their recent publication the activity of N-succinyl gramicidin and of N-O-succinylgramicidin. They reported N-succinyl gramicidin to be active with a significantly different channel mean lifetime than unmodified gramicidin and N-O-succinyl gramicidin to be inactive presumably as the dianion. From this it was concluded that the dimerization of gramicidin occurs under these conditions by an "end-to-end" association. One of several end-to-end possibilities enumerated by the authors would place the N-terminal succinyl group at the interface.

In this work, however, a high activity of N-O-succinyl gramicidin (Fig. 1*c*) at low pH was found. At the lowered pH of the electrolyte solution we also were able to detect a charge effect on the single-channel conductance arising from a deprotonated group at the mouth of the pore. To clarify the results of Bradley *et al.* (1978) and those of our experiments, an O-pyromellityl-N-succinyl gramicidin (Fig. 1*d*) was synthesized. In a previous paper by Apell *et al.* (1977), it was shown that the threefold negatively charged group of the pyromellitic residue on the ethanolamine end of the molecule cannot be titrated between pH 3–8 as is possible for the succinic groups, as shown above.

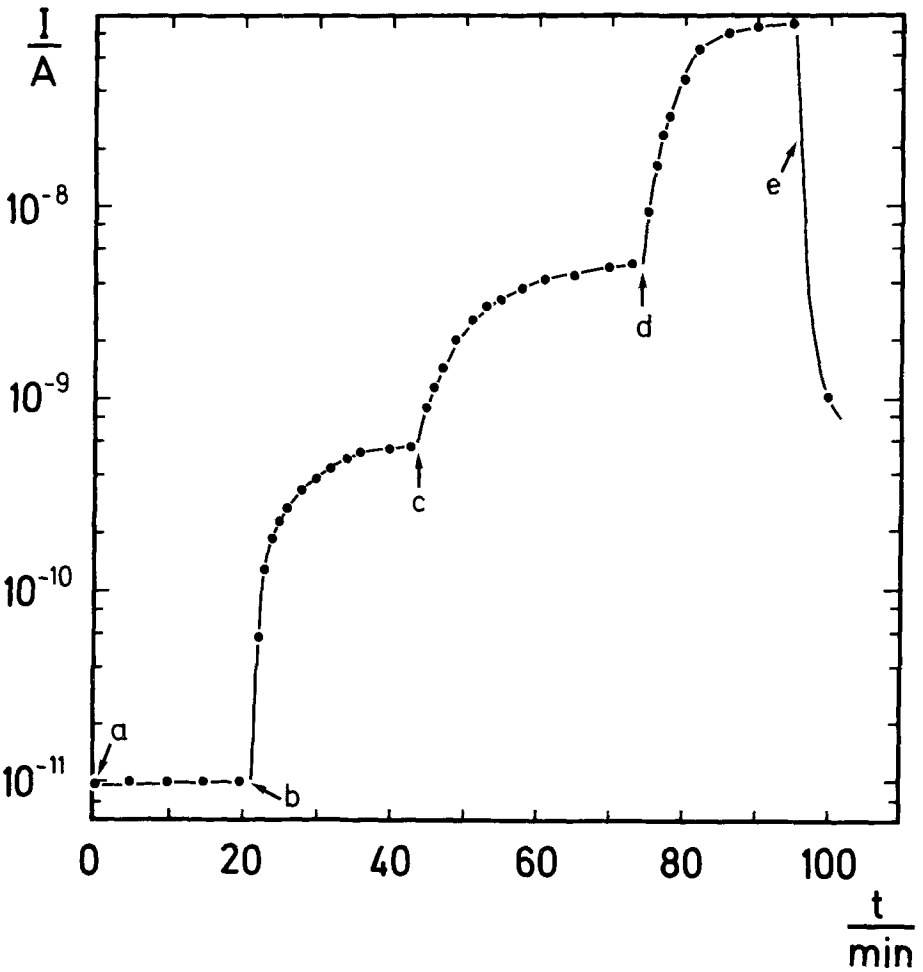

Fig. 3. Membrane current I after asymmetric and symmetric addition of N-succinyl-O-pyromellityl gramicidin. The membrane was formed from PC 16:4 CH_3 in n-decane. Both aqueous solutions obtained 1 M CsCl, 2×10^{-4} M citric acid, pH 5, $T = 25$ °C. The applied voltage was 10 mV. (*a*): At time $t = 0$, 20 µl of a 10^{-6}-M methanolic solution of N-succinyl-O-pyromellityl-gramicidin were added to one aqueous compartment to give a concentration of 2×10^{-9} M. (*b*): At time $t = 20$ min, the same amount was added to the other compartment. (*c*): At $t = 42$ min, the concentration of the N-succinyl-O-pyromellityl gramicidin was doubled. (*d*): At $t = 75$ min, 10 µl of a 10^{-5} M solution of N-succinyl-O-pyromellityl-gramicidin was added to both compartments. (*e*): At $t = 95$ min, 10 µl of a 0.2 M NaOH was added to both compartments to increase the pH to approx. 6

Experiments in the presence of O-pyromellityl-N-succinyl gramicidin show a strong pH dependence of the macroscopic conductance as shown above for N-succinyl gramicidin (Fig. 3). The conductance increased at low pH if the substance is applied to both sides of the membrane. The application to one side does not cause a remarkable conductance

change of the membrane. Increasing the pH leads to a strong reduction of the membrane conductance (Fig. 3).

This experiment leads to the following conclusion: Because of the three negative charges, the molecule cannot penetrate the membrane and form conducting channels. Only when it applied to both sides of the membrane are active channels formed. Increasing the pH leads to an inactivation; i.e., when the N-succinyl group is deprotonated, the dimerization process becomes less probable. With single-channel experiments in the presence of O-pyromellityl-N-succinyl gramicidin, it can be checked whether the interpretation of the results of the macroscopic conductance is reasonable or not. Apell *et al.* (1977) were able to show that strong charge effects at the mouth of the pore for O-pyromellityl gramicidin increase the single channel conductance compared to unmodified gramicidin A. A very similar behavior can be observed for O-pyromellityl-N-succinyl gramicidin (Fig. 4). This figure shows clearly that

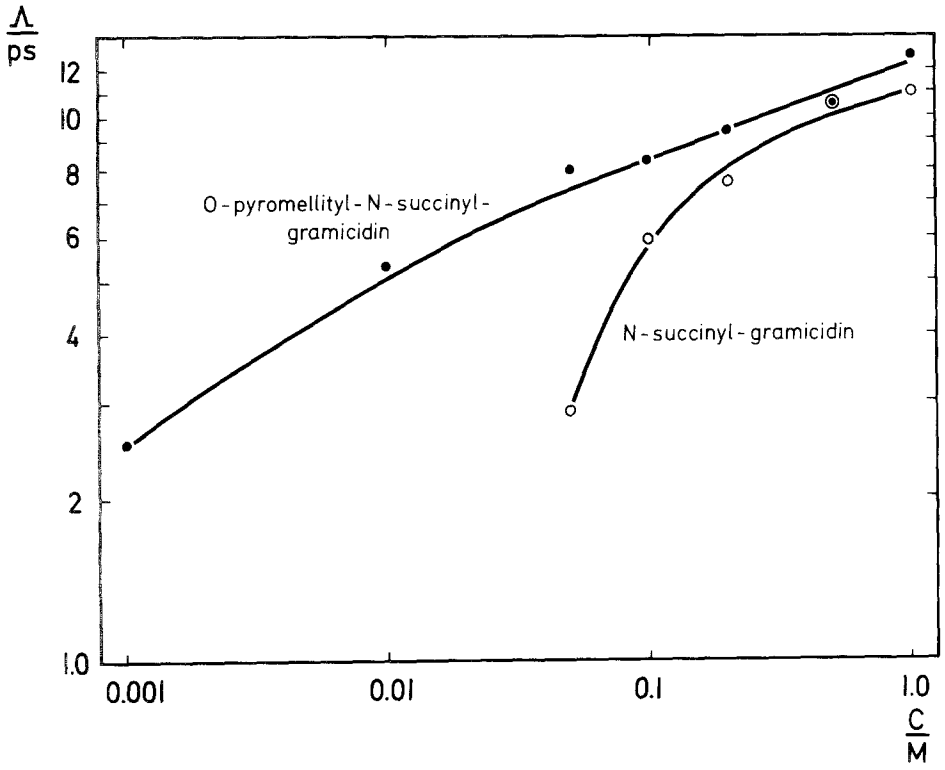

Fig. 4. Dependence of the single-channel conductance on the concentration of the transported ion in the presence of N-succinyl gramicidin and of O-pyromellityl-N-succinyl gramicidin. Electrolyte CsCl; lipid monooleine/*n*-hexadecane; 2% wt/vol; pH = 4.5. Applied voltage, 100 mV.

at low ionic strength a pronounced charge effect at the mouth of the pore occurs. At 50 mM the single-channel conductance is increased by a factor of 3 compared to N-succinyl gramicidin. For N-succinyl gramicidin no charge effects can be observed over the measured ionic concentration range (Table 1).

Summarizing the results obtained with the different succinyl-gramicidin derivatives allows the following conclusion with respect to the structural nature of the gramicidin channel. The pH dependence of the N-succinyl-gramicidin derivatives shows clearly that an association of two monomers can only occur when the carboxylic group at the N-terminal of the molecule is protonated. This gives further evidence that the single stranded $\beta(\pi_{L,d})$-helix is the active channel in the membrane. The different types of the double stranded helix, however, should be present independent of the protonated or deprotonated state of the succinic group. The absence of these structures can also be concluded from the pH dependence at the single-channel level where it is found that the single channel conductance of N-succinyl gramicidin is invariant to changing the pH of the electrolyte. No charge effect on the single-channel conductance can be observed when the N-succinyl group is deprotonated. But in this state the molecule seems to be inactive (Fig. 2). From these data it can be said definitely that the N-succinyl residue must be located in its protonated form in the middle of the membrane.

The experiments with O-pyromellityl-N-succinyl gramicidin reduce the possibilities for the structure of the channel to one possible structure. Upon adding the O-pyromellityl-N-succinyl gramicidin to both sides of the membrane at low pH, a high channel formation rate can be found (Fig. 3). On increasing the pH, the activity is reduced dramatically. Furthermore, a large charge effect of the single-channel conductance over the whole measured pH-range can be observed. This indicates the same behavior of the pyromellityl residue as shown previously (Apell *et al.*, 1977); namely, the three negative charges at the mouth of the pore cannot be removed by lowering the pH to values of $pH \geqq 4.0$. Therefore, it is evident that the pyromellityl residue must be located in the membrane interface and that the critical part of the molecule is the titrable COOH group of the succinic residue of the gramicidin derivatives. Also, an active channel can be formed when the succinic group is protonated and therefore can be located in the interior of the membrane.

Two consequences for the structure of the gramicidin channel follow from these considerations.

a) The formyl group on the head of the molecule can be replaced by a succinic group.

b) The proposed "end-to-end" association of the N-succinyl gramicidin can be reduced to a head-to-head association by the experiments with the O-pyromellityl-N-succinyl gramicidin. As mentioned above, the threefold negatively charged group must be located in the interface, so that this part of the molecule cannot give a contribution to the dimerization process.

Relaxation Experiments

The single-channel and steady-state experiments, described in the first part of the paper showed that it is possible to replace the formyl group by a titrable succinic residue. Besides a changed single-channel conductance compared with gramicidin A, a different mean life time of the N-succinyl derivatives was also observed. This observation is also reflected in the results of the relaxation experiments, which are presented in the following. As shown previously (Bamberg & Läuger, 1973), the dimerization of gramicidin in a membrane can be expressed by

$$G + G \underset{k_D}{\overset{k_R}{\rightleftharpoons}} G_2 \tag{1}$$

k_R and k_D are the association and dissociation rate constants of the reaction. The equilibrium between monomers (G) and dimers (G_2) in the membrane can be changed by a voltage jump. The analysis of the current relaxation into a new equilibrium after a voltage jump allows determination of the rate constants, using the equation (Bamberg & Läuger, 1973)

$$\frac{1}{\tau} = 4k_D + \sqrt{\frac{k_R k_D \cdot \lambda_\infty}{\Lambda \cdot L}}$$

where τ is the relaxation time, λ_∞ the new stationary current after voltage jump, Λ the single channel conductance, and L the Avogadro's number.

The experiments showed that N-succinyl gramicidin behaves in the same manner as does normal gramicidin and that rate constants can be determined.

Figure 5 shows a typical current relaxation of a N-succinyl gramicidin doped membrane which can be described by a single exponential function. As published with normal gramicidin (Bamberg & Läuger, 1973),

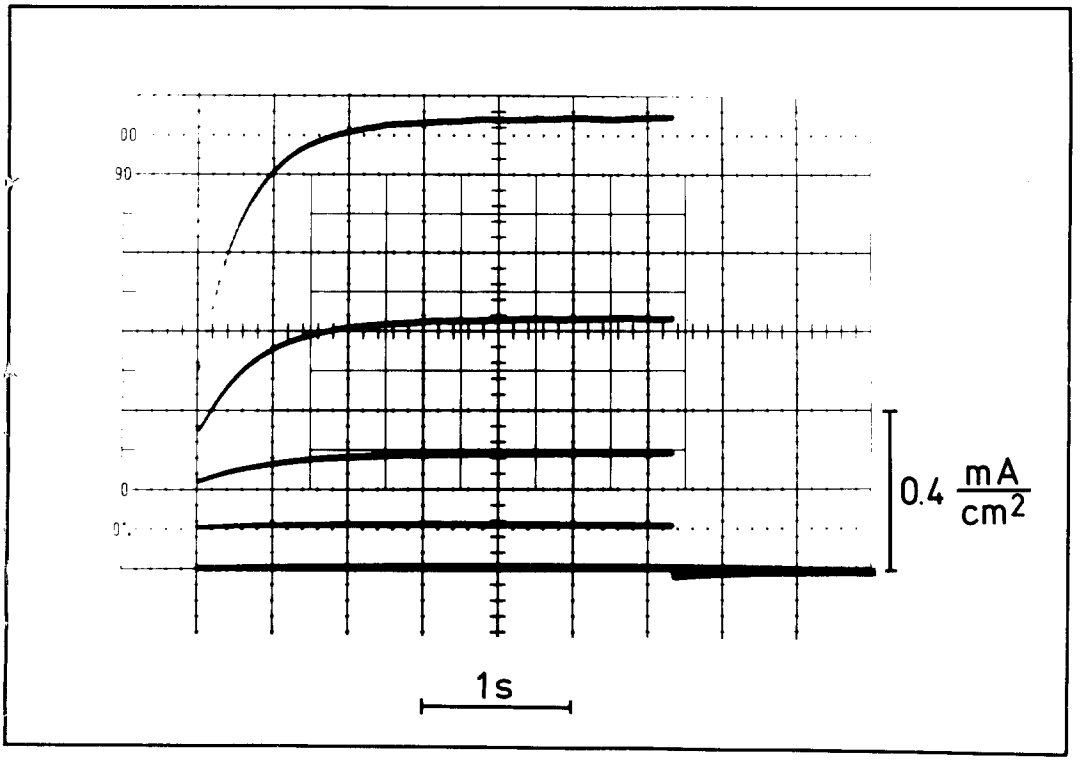

Fig. 5. Relaxation of the membrane current after a voltage jump in presence of N-succinyl gramicidin. 1 M CsCl dioleoyl-lecithin/*n*-decane 1% wt/vol; applied voltage, 50, 100, 150, and 200 mV; $T=25°$; pH=4.0

there is a qualitative agreement of the mean life time of the single channels and the inverse of the dissociation rate constant k_D for N-succinyl gramicidin channels also. This is a further fact which allows the conclusion that the basic properties of the gramicidin channel are not changed by the modification of the molecule.

Table 2 contains the results obtained by relaxation experiments with N-succinyl gramicidin. For comparison, the data for normal gramicidin A are also listed.

A surprising result is that the activation energies of the dimerization process are more or less the same for the N-succinyl gramicidin as they were obtained for the unmodified gramicidin A. The accuracy determination of the activation energies is ± 2 kcal/mol.

The head-to-head association of the gramicidin A is effected by 6 hydrogen bonds between the two monomers, according to Urry *et al.* (1977). With a determined activation energy of the dissociation rate constant k_D of 17–18 kcal/mol the dissociation energy of one hydrogen

Table 2. Evaluation of the relaxation experiments in presence of N-succinyl gramicidin[a]

N-succinyl gramicidin					Gramicidin A			
T °C	Λ pS	k_R $cm^2mol^{-1}sec^{-1}$	k_D sec^{-1}	K cm^2mol^{-1}	Λ pS	k_R	k_D	K
10°	–	1.9×10^{14}	1.0	1.9×10^{14}	15	2.3×10^{13}	0.25	9×10^{13}
25°	3.7	1.3×10^{15}	5.0	2.7×10^{14}	30	20×10^{13}	1.6	12.5×10^{13}
40(35°)	7.4	6.1×10^{15}	8.0	3.4×10^{14}	60	68×10^{13}	4.5	15×10^{13}
	E_Λ kcal mol^{-1}	E_{k_R} kcal mol^{-1}	E_{k_D} kcal mol^{-1}	E_k kcal mol^{-1}	E_Λ kcal mol^{-1}	E_{k_R} kcal mol^{-1}	E_{k_D} kcal mol^{-1}	E_k kcal mol^{-1}
	8.6	20.3	16.6	3.4	7.3	20	17	2.9

[a] $C=1$ M CsCl; $T=25°$; pH=4.0. Applied voltage: 150 mV; lipid: dioleoyllecithin/*n*-decane, 1% wt/vol. k_R=recombination rate constant; k_D=dissociation rate constant; K=equilibrium constant; E_Λ=activation energy for ion transfer through the channel; E_{k_R}, E_{k_D}, E_K=activation energies related to the recombination and dissociation rate constant and the equilibrium constant. The values for gramicidin A were taken from a previous publication (Bamberg & Läuger, 1974) at 10, 25, and 40 °C. The values for N-succinyl gramicidin are measured at 10, 25 , and 35 °C.

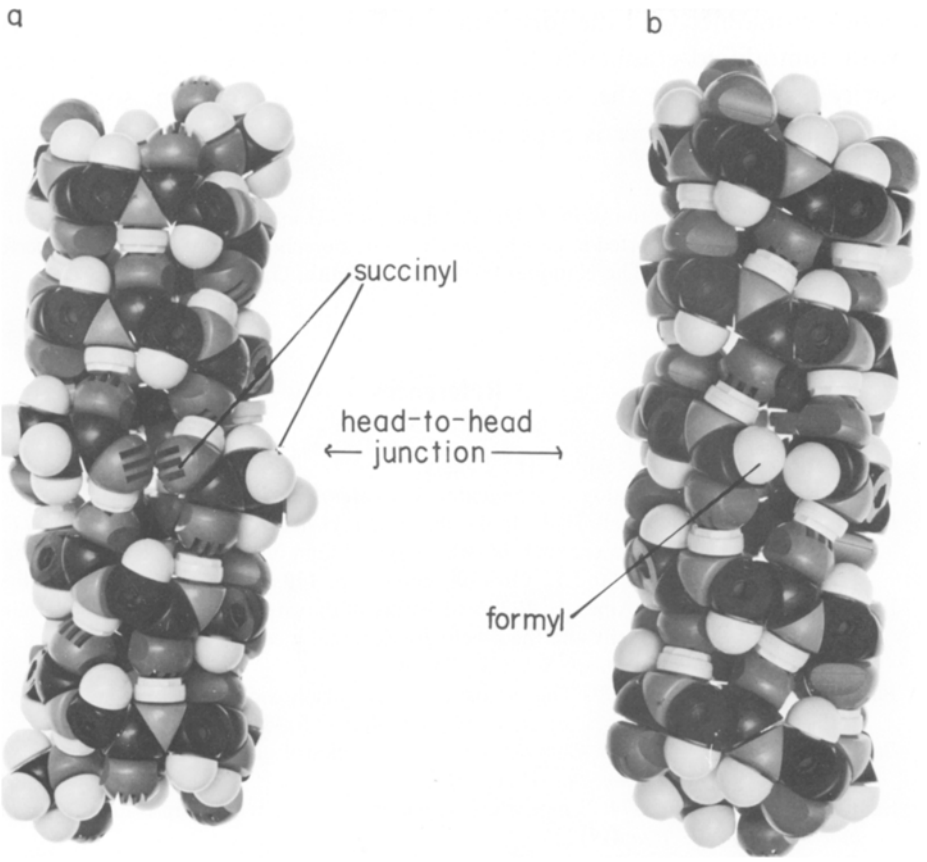

Fig. 6. Corey-Pauling-Koltun molecular models of the head-to-head hydrogen bond dimerization $\beta(\pi_{LD})$-helix of N-succinyl desformyl gramicidin (*a*) and of the unmodified gramicidin (*b*). Both structures have six hydrogen bonds effecting dimerization. Note how compactly the protonated succinyl moiety hydrogen bonds; it effectively occupies the role of an additional residue. Some destabilization can be expected due to the juxtaposition of the OH oxygens of the carboxyl group

bond is in the range of 3 kcal/mol, a quite reasonable value for a hydrogen bond in the apolar interior of a membrane.

The dissociation energy of 16–17 kcal/mol for N-succinyl gramicidin again underlines a dimerization process comparable to that of gramicidin A. With the limitations inherent in the determination of the activation energy for dissociation, it is not possible to determine how the sixfold reduction in mean life time, reported by Bradley *et al.* (1978), separates into energies and entropies of activation for dissociation, as the factor of six, if reflected solely by activation energy, would constitute a difference of only 1 kcal/mol. As shown in Fig. 6*a* with CPK models, two N-succinyl gramicidin molecules can associate head to head in a

compact manner with the formation of six hydrogen bonds as can occur with unmodified gramicidin (*see* Fig. 6*b*). As the OH oxygens are necessarily juxtaposed in the N-succinyl gramicidin association, some destabilization of the dimer is expected.

The authors wish to thank Prof. Dr. P. Läuger for his interest and helpful discussions. This work has been supported in part by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 138) and The National Institutes of Health, Grant No. HL-11310.

References

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

Bamberg, E., Alpes, H., Apell, H.-J., Janko, K., Kolb, H.-A., Läuger, P., Gross, E. 1977*a*. Studies on the gramicidin channel. *In:* FEBS Symposium on Biochemistry of Membrane Transport. G. Semenza and E. Carafoli, editors. p. 179, Springer Verlag, Heidelberg

Bamberg, E., Apell, H.-J., Alpes, H. 1977*b*. Structure of the gramicidin channel. Discrimination between the $\pi_{L,D}$-helix and the β-helix by electrical measurements with lipid bilayer membranes. *Proc. Nat. Acad. Sci. USA* **74**:2402

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

Bamberg, E., Läuger, P. 1973. Channel formation kinetics of gramicidin A on lipid bilayer membranes. *J. Membrane Biol.* **11**:177

Bamberg E., Läuger, P. 1974. Temperature dependent properties of gramicidin A channels. *Biochim. Biophys. Acta* **419**:223

Bradley, R.J., Urry, D.W., Okamoto, K., Rapaka, R. 1978. Channel structures of gramicidin A: Characterization of succinyl derivatives. *Science* **200**:435

Hladky, S.B., Haydon, D.A. 1972. Ion transfer across lipid membranes in presence of gramicidin A. Studies on the unit channel conductance. *Biochim. Biophys. Acta* **274**:294

Janko, K., Benz, R. 1977. Properties of lipid bilayer membranes made from lipids containing phytanoic acid. *Biochim. Biophys. Acta* **470**:8

Läuger, P., Lesslauer, W., Marti, E., Richter, J. 1967. Electrical properties of bimolecular phospholipid membranes. *Biochim. Biophys. Acta* **135**:20

Sauvé, R., Bamberg, E. 1978. $1/f$ noise in black lipid membranes induced by ionic channels formed by chemically dimerized gramicidin. *J. Membrane Biol.* **43**:317

Urry, D.W. 1971. The gramicidin A transmembrane channel: A proposed $\pi_{L,D}$-helix. *Proc. Nat. Acad. Sci. USA* **68**:672

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

Urry, D.W., Goodall, M.C., Glickson, J.D., Mayers, D.F. 1971. The gramicidin A transmembrane channel: Characteristics of head to head dimerized $\pi_{(L,D)}$ helices. *Proc. Nat. Acad. Sci. USA* **68**:1907

Veatch, W.R., Fossel, E.T., Blout, E.R. 1974. The conformation of gramicidin A. *Biochemistry* **13**:5249