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Ionic channels induced by surfactin in planar lipid bilayer membranes

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Surfactin is a lipopeptide produced by certain strains of *Bacillus subtilis* and has potent surface activity. Here, we present the first results showing that ion-conducting pores can be formed by surfactin in artificial lipid membranes. With a low aqueous concentration of surfactin (1 μM) and a restricted membrane area ($5 \cdot 10^{-5}$ cm^2) we observed conductance jumps that indicate the formation of individual ionic channels in the presence of K^+, Rb^+, Cs^+, Na^+ or Li^+ chlorides. Although for every salt concentration (C_i), the distribution in amplitude of the conductance steps (Λ_i) may be rather broad, there is always a step amplitude which is more frequent than the others. In addition, the channels corresponding to this most frequent step amplitude are the longest in duration. For $C_i = 1$ M, the cationic selectivity sequence deduced from these most frequent events is $K^+ > Rb^+ > Na^+ > Cs^+ = Li^+$ with respective values for Λ_i^M: 130, 110, 80 and 30 pS. In KCl solutions Λ_{KCl}^M increases as a function of C_i for low C_i, and shows a plateau for $C_i > 0.5$ M. When measured on larger area membranes (10^{-2} cm^2) with 1 M solutions of the monovalent salts KCl, NaCl, RbCl and CsCl or the divalent salt $CaCl_2$, the macroscopic low voltage conductance (G_0) increases with a slope of 2 on a log-log plot as a function of surfactin concentration. These results demonstrate that surfactin produces selective cationic channels in lipid bilayer membranes and suggest that at higher salt concentration, a dimer is involved in this functional channel-forming process.

Introduction

Surfactin is a lipopeptide secreted by certain strains of *Bacillus subtilis*. It has potent surface activity, lowering the surface tension of distilled water from 72 to 27 mN/m [1]. This molecule has been shown to lyse erythrocytes, burst certain protoplasts and exhibits some antibiotic ability presumably as a result of interactions with membranes [2–4].

Surfactin is composed of a fatty acid (3-hydroxy-13-methyltetradecanoic) with a hydrophilic ring of seven amino acids in the order: L-Glu-L-Leu-D-Leu-L-Val-L-Asp-D-Leu-L-Leu [1,5]. This structure is very similar to that of the iturin group of antibiotics which are also produced by several strains of *B. subtilis* [6]. However, three major differences are observed. First, even though the iturins also have seven amino acids arranged in a ring, surfactin is closed by a β-amino acid instead of by a 3-hydroxyl group on the fatty acid. Second, surfactin does not contain any tyrosine, which is common to all of the iturins. Finally, surfactin, due to the aspartate and glutamate residues, bears two negative charges at normal pH.

The activity of some of the iturins is similar to that observed for surfactin, with the ability to lyse protoplasts and erythrocytes, but the iturins modify also the membrane permeability of *Saccharomyces cerevisiae* [7–10] and can produce ion-conducting pores in artificial lipid membranes [11–13]. It is known that methylation of the tyrosine residue modifies the ion conductance [14]. Therefore, the tyrosine is apparently involved in the formation of channels. Since surfactin shares the general structure and many characteristics of the iturins but differs completely with respect to amino acids, we thought it would be of interest to study its pore-forming behaviour.

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Materials and Methods

(A) Bacterial strain and surfactin isolation

The surfactin was obtained by growing *B. subtilis* ATCC 21332 in batch culture in a defined mineral salt medium [15]. After reaching the stationary phase, the cells were removed by centrifugation, the pH of the broth lowered to 2, the precipitate collected and extracted with chloroform. Then, the chloroform was evaporated and the residual solids were resuspended in distilled water at pH 9. An analysis of the amino acids in the solids confirmed the presence of surfactin at about 90% purity based on total protein and there was no trace of tyrosine.

(B) Lipid bilayer experiments

Lipid bilayers were formed with a solution of glyceryl monooleate (25 mg/ml) (Sigma Chemical Co., St. Louis, MO) dissolved in *n*-decane. The membrane was painted across a hole made in a Teflon disc which separated two glass compartments each holding 20 ml or 4 ml of aqueous solution [16–18].

The membrane surface was 0.005 mm^2 for single-channel experiments and 1.0 mm^2 for macroscopic conductance. The surfactin was added to the aqueous phase on both sides of the membrane. Addition of surfactin to only one side did not appear to change the single-channel nor the macroscopic conductance behavior. The latter appeared to depend on the total amount of surfactin added, whether on one or both sides of the membrane.

Individual channel conductances were measured by applying voltage across the membrane with a pair of Ag/AgCl electrodes dipped in the aqueous phases and measuring current with an operational amplifier in a current amplifier configuration with a feedback resistance of 10^9 ohms [18]. The signal was monitored on a storage oscilloscope and recorded on a strip chart recorder (Omnigraphic, Houston Instruments, model 3000) or an FM tape recorder (Racal Recorders Ltd).

For macroscopic membrane conductance measurements, a 0.01 Hz, alternating polarity 5 mV voltage was applied as above to the membrane. The current was measured with a Keithley electrometer No. 602 (Keithley Instruments, Cleveland, OH) connected in series with the membrane. The electrometer being used in the current mode, the current is obtained from V_k, the voltage measured across the known resistor R_k. V_0 being the applied voltage, the membrane conductance G_0 is given by: $G_0 = (1/R_k)(V_k/(V_0 - V_k))$. R_k is chosen (factors of 10) to be comparable to the membrane resistance.

Results

(A) Single-channels experiments

When surfactin was added at low concentration in the aqueous phase (0.5 to 2.0 μM) with small area membranes, variations in the membrane current ex-

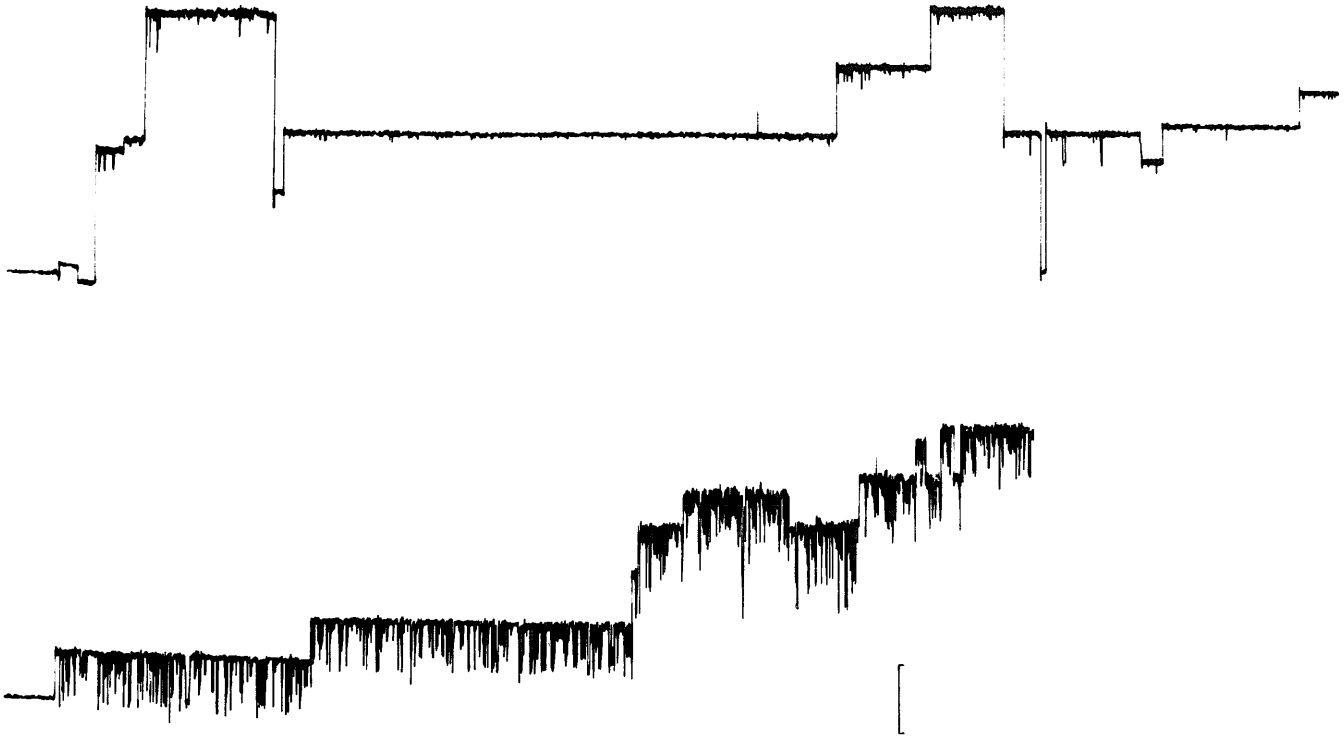

Fig. 1. Conductance steps caused by addition of surfactin at 1.4 μM final concentration to the aqueous phase bathing a lipid bilayer membrane made with glyceryl monooleate 2.5% in decane over a hole of 0.005 mm^2. The applied voltage was 80 mV. Upper trace: in 0.95 M KCl (the scale for the first 45 s is 5 pA/unit), lower trace: in 0.2 M KCl. The vertical bar indicates 10 pA and the horizontal one, 10 s.

hibited a stepwise behaviour, indicative of opening and closing of individual channels. A typical recording is shown in Fig. 1. Although the channels remain open most of the time, there are very fast closings. These closings appear to be more frequent at a low salt concentration (KCl 0.2 M) as compared to a high salt concentration (0.95 M). In addition, the analysis of the conductance steps reveals that the channels remain open for a longer time period in 0.95 M KCl than in 0.2 M KCl (average value of 16 min as compared to 3.6 min).

Other than the fast closings from an open state in general, few channel closings were observed. Thus, only upward-directed steps were used in calculating the distribution histograms of single-channel increments. Fig. 2 shows histograms of such conductance steps for different KCl concentrations. Although the conductance steps covered a fairly large range of values, we were able to determine for each KCl concentration a most frequent conductance. Also, it can be seen that whatever the concentration, the percentage of steps higher than the most frequent one is less than the percentage of those lower.

In addition, since the channel closings did not occur frequently and the number of channels appearing was of the order of one per minute, it was possible over a period of 5 to 20 min, to measure the time a given channel remained open. Fig. 2 also represents the average percentage of the observation time period that

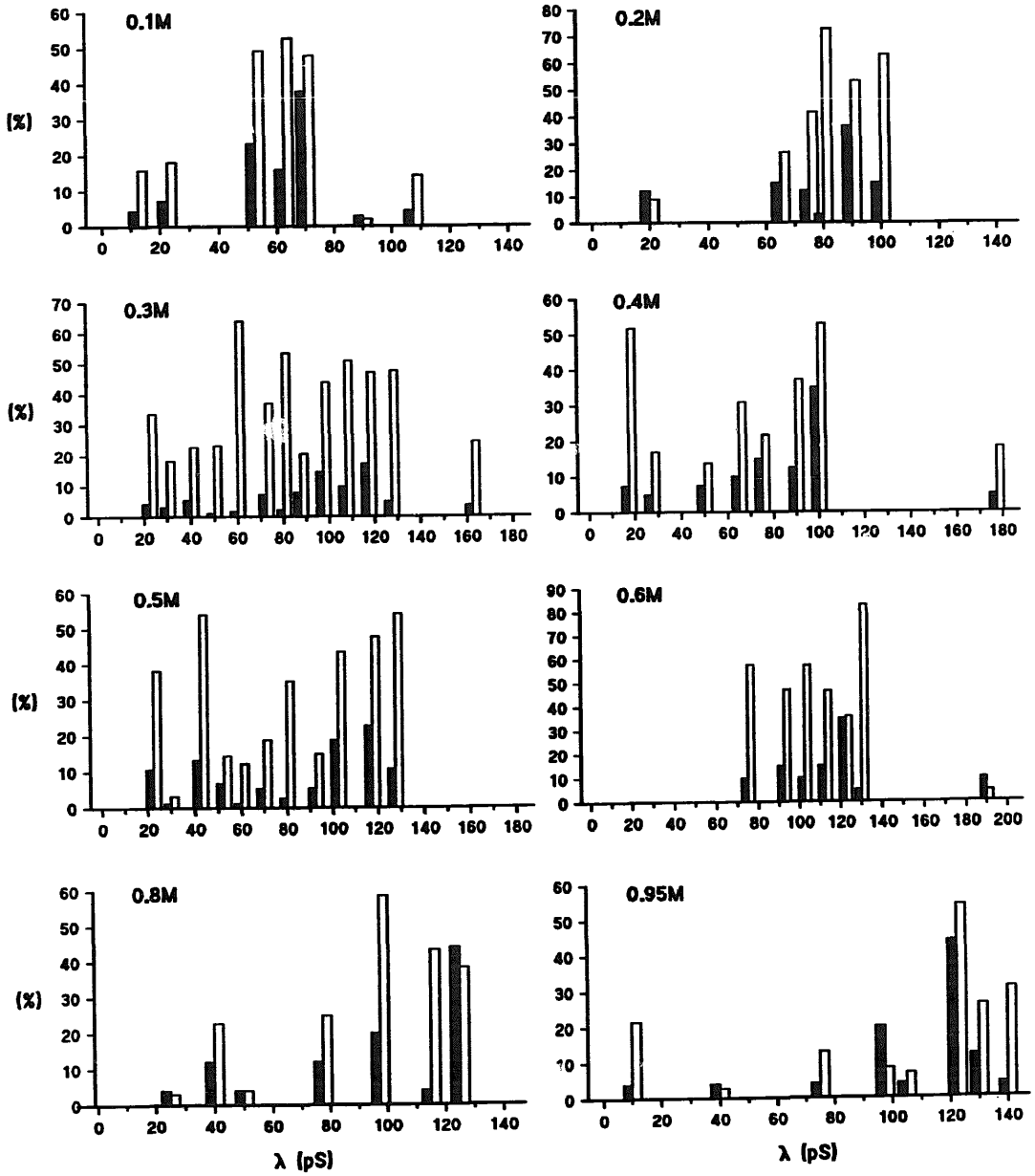

Fig. 2. Closed bars: histograms of conductance steps in KCl at different salt concentrations. Only upward steps were considered. Open bars: percentage of the total time of observation conductance steps of a given value remained on.

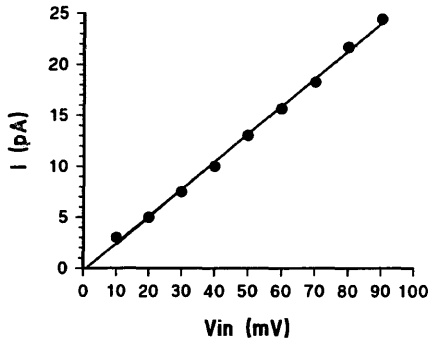

Fig. 3. Relationship between the current through a single channel and the voltage applied across the membrane. The aqueous phases contained 1 M NaCl and surfactin at 0.6 μM.

channels of a given conductance remained open. It can be seen that for the great majority of cases the most frequent conductance steps are also those lasting for the longest time period.

The conductance of the pores formed by surfactin are not voltage-dependent since the individual channel current is directly proportional to the applied voltage as can be seen in Fig. 3. Also, the channels appear to be symmetrical, since switching the voltage, for example, from -50 mV to $+50$ mV produced an equivalent current of opposite sign across the membrane.

For Rb^+, Cs^+, Na^+ and Li^+ at 1 M chloride salt concentration, the histogram for the conductance steps was very similar to the one shown for KCl 0.95 M in Fig. 2 with a clearly identified most frequent amplitude. The cationic selectivity sequence, as derived from the most frequent single-channel conductance at 1 M salt concentration, is $K^+ > Rb^+ > Na^+ > Cs^+ = Li^+$ with respective conductance values Λ_i^M: 130, 110, 80 and 30 pS. This selectivity pattern is represented in Fig. 4 (closed bars) which shows the ratio of the conductance for the most frequent step for a given ion relative to that obtained in KCl.

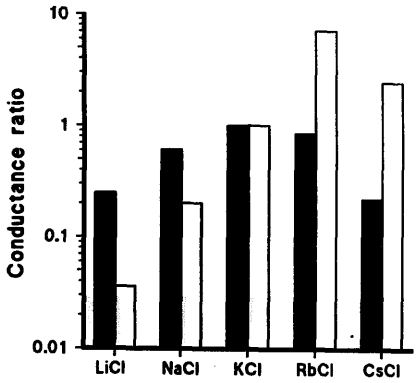

Fig. 4. Selectivity sequence ratio with respect to KCl for different salts at 1 M concentration in the aqueous phases. Closed bars represent the ratio obtained in single-channel experiments, $\Lambda_i^M/\Lambda_{KCL}^M$, at low surfactin concentration. Open bars represent macroscopic conductance ratio, $G_{0(i)}/G_{0(KCL)}$, obtained with surfactin at 2 μM concentration.

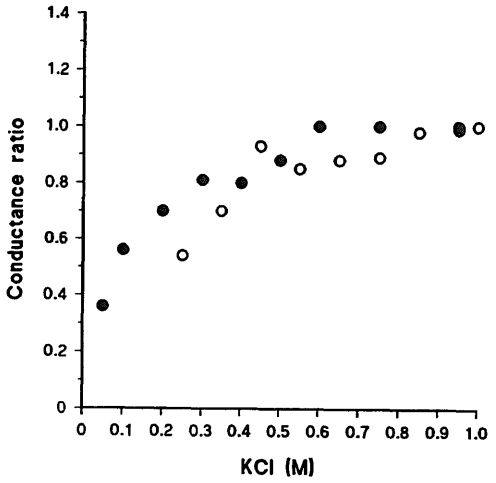

Fig. 5. Conductance induced by surfactin as a function of KCl concentration. Filled circles represent the ratio of the conductance of the most frequently opened single channel at a given concentration (Λ_{KCL}^M) relative to that at 0.95 M. These data are directly obtained from the histograms shown in Fig. 2. Open circles represent in an identical fashion initial (1 min) macroscopic conductance G_0 induced by successive additions, to a membrane already equilibrated with surfactin (1.6 μM) in 0.25 KCl, of small volumes of KCl 3 M to yield the indicated KCl concentration (average of 3–5 membranes).

On the other hand, if we now consider in Fig. 2 the single-channel conductance values for KCl that occur most frequently (Λ_i^M) and plot the ratio of the conductance observed at a given KCl concentration over that observed at 0.95 M, we see (Fig. 5 filled circles) that the single-channel conductance increases as a function of KCl concentration until it reaches a plateau for concentrations higher than 0.5 M.

(B) Macroscopic conductance experiments

When surfactin is added at a concentration ranging from 0.2 to 20 μM in the aqueous phase on both sides of large area membranes (10^{-2} cm^2), we observe that the macroscopic low voltage conductance, G_0, increases gradually and tends towards a steady-state value (Fig. 6). Analysis of the data for various ions at 1 M concentration and for different ion concentrations for KCl reveals an exponential function for the increase in G_0, the time constant varying from 10 to 20 min. Fig. 7 shows on a log-log scale the steady-state values obtained for G_0 with KCl, NaCl, RbCl and CsCl salt solutions at 1 M concentration as a function of surfactin concentration. Each point represents a value obtained with a given membrane 20–40 min after adding surfactin so a stable level of conductance had been reached. It is important to note that the slopes of linear regressions of the experimental data all give a value close to 2 (2.0, 2.18, 1.96 and 2.0 for KCl, NaCl, RbCl and CsCl, respectively). Hence, we may infer that a dimer is involved in the pore-forming process. A macroscopic selectivity ratio relative to KCl can be extracted from these data. Taking the ratio of the conductance

Fig. 6. Temporal increase of the macrosopic conductance G_0 after addition of surfactin (1.0 μM) to the aqueous phase. The trace represents the voltage recorded by the Keithley electrometer (across a 10^8 Ω resistor) following application of a 10 mV peak to peak alternating voltage across the membrane in series with the Keithley resistor. The salt was NaCl at 0.5 M. At the arrow, the surfactin was increased to 2.6 μM. The vertical bar represents 0.6 mV and the horizontal bar, 1 min.

obtained at 2 μM surfactin for CsCl, RbCl, NaCl and LiCl, resulted in the pattern shown in Fig.4 (open bars). This pattern can be compared in the same figure with that obtained for single conductance steps (closed bars). It can be seen that in addition to the inversion of KCl with RbCl and the increased selectivity in favor of CsCl with respect to LiCl, the magnitude of the selectivity deduced from G_0 measurements is greatly enhanced.

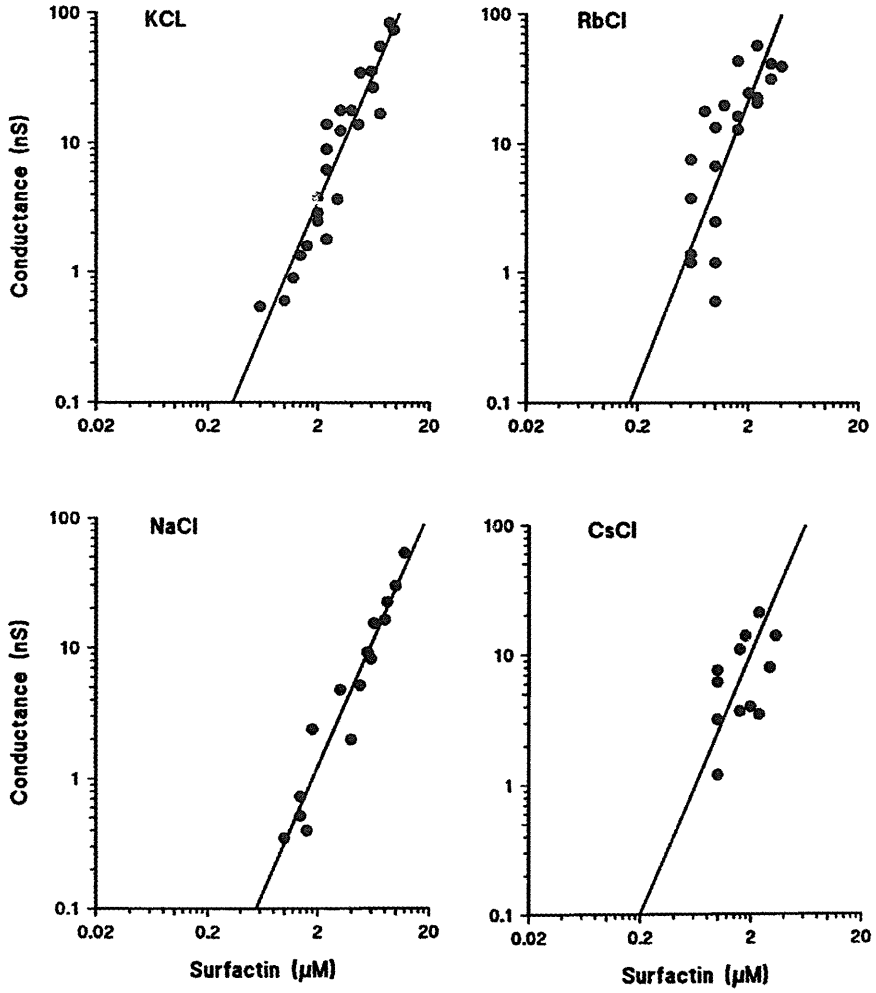

Fig. 7. Macroscopic conductance G_0 as a function of surfactin concentration in different salts at 1 M concentration. The membrane was made with glyceryl monooleate 2.5% in *n*-decane over a hole of 1.0 mm^2 and the applied voltage was 80 mV.

Figs. 8 and 9 show the G_0 data as a function of surfactin concentration for different KCl and NaCl concentrations. We can observe that the slopes of conductance vs. surfactin concentration decrease for lower salt concentration. Indeed, for KCl at 1 M and 0.75 M, slope values are, respectively, 1.995 and 2.186, while they are 1.266 and 1.415 for 0.5 M and 0.25 M KCl, respectively. For NaCl, the slope is 1.96, 0.996 and 1.03 at 1 M, 0.5 and 0.25 M, respectively. The above conductance data can be replotted as a function of salt concentration for various surfactin concentration levels (0.2, 0.6, 1.0, 1.4 and 2.0 μM). As can be seen in Fig. 10, the result shows a striking phenomenon either for KCl or NaCl solutions, the conductance reaching a sharp maximum at 0.5 M.

In order to gain some insight into the mechanisms responsible for this behavior of the conductance as a function of salt concentration, we conducted another series of experiments where instead of increasing surfactin concentration for a given salt concentration, we started with a given surfactin concentration and a low KCl concentration (0.25 M), then increasing the KCl concentration to the desired value by adding appropriate volumes of 3 M KCl. In these experiments, surfactin (1.6 or 0.8 μM) was added to a 0.25 M KCl solution and the conductance was followed until a steady level was reached indicating that the average number of molecules or functional pores in the membrane was stable. Then the KCl concentration was increased by adding the proper volume of 3 M KCl. The conductance increased in a two-step fashion: a rapid increase which was over within 1 min and a longer one lasting around 20 min. The first one probably reflects the single-channel conductance increase due to increased KCl concentration (see Fig. 5, closed circles) while the slower one is most likely associated with an increase in the number of channels from molecules already present in the membrane, induced by the increase in aqueous

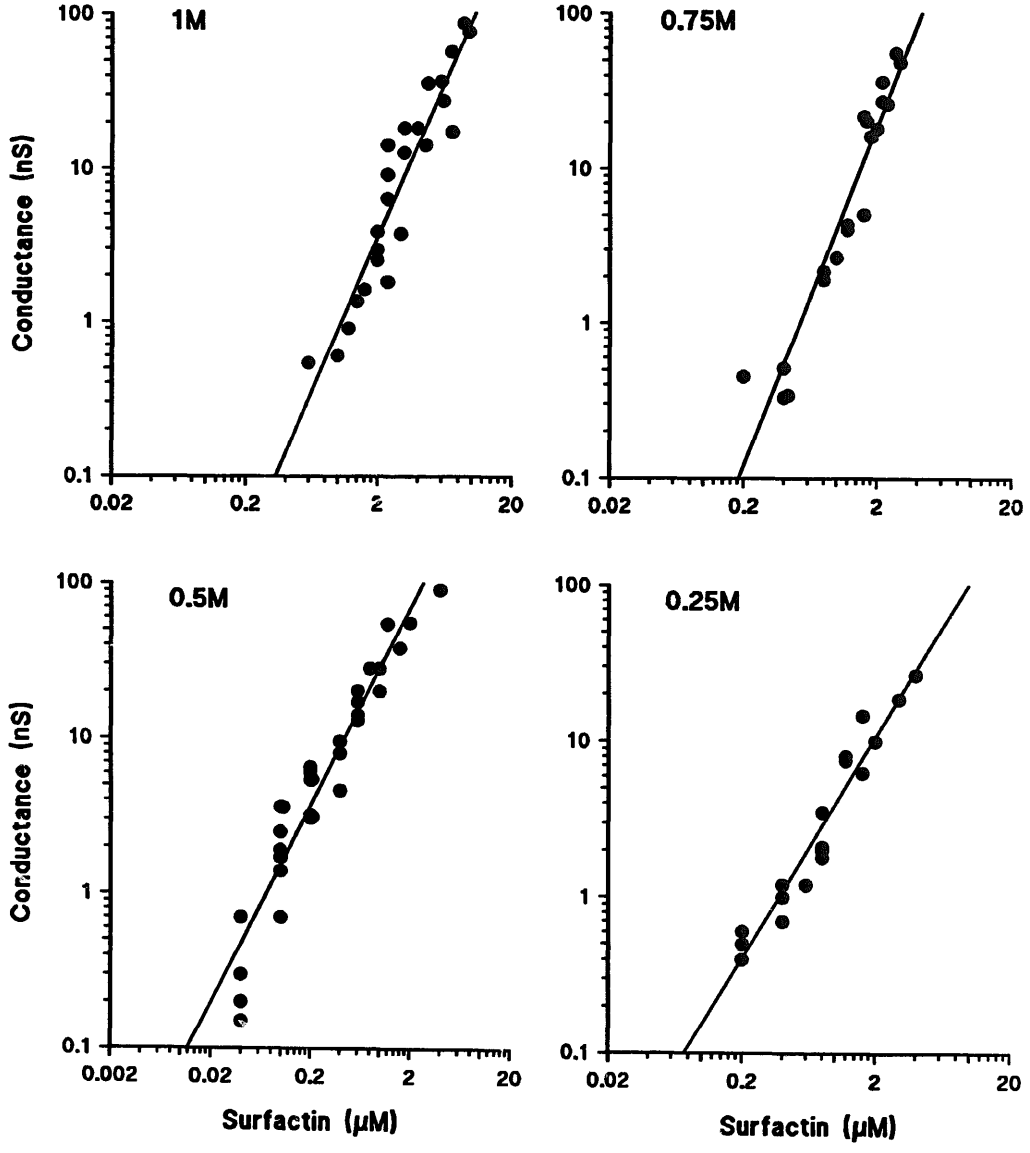

Fig. 8. Macroscopic conductance G_0 as a function of surfactin concentration for different concentrations of KCl solutions.

ion concentration. The results for the initial increase (1 min) are presented in Fig. 5 (open circles) in the same manner as for the single-conductance steps, as the ratio of the conductance at a given KCl concentration to that at the maximum KCl concentration (1 M). It can be seen that the macroscopic conductance behavior is now very similar to that of the single-channel conductance and no longer shows a maximum. On the other hand, the longer term conductance which is shown in Fig. 11 shows a hyperbolic increase which is steeper from 0.25 to 0.5 M with a slope which is close to that seen in Fig. 10 for the same range of concentration. From 0.5 to 1 M, again in contrast with Fig. 10, the conductance still increases but with a slope close to 2.

In order to further study the stoechiometry of interactions of ions with surfactin, we studied the conductance induced by surfactin in the divalent salt $CaCl_2$. The results are shown in Fig. 12 where we have plotted the membrane conductance as a function of surfactin concentration for three different salt concentrations (1.0, 0.75 and 0.6 M). No data could be obtained at a lower $CaCl_2$ concentration since the membrane broke very rapidly after formation. Interestingly, we observe for this divalent salt that the slopes of linear regression for the experimental data do not change significantly as a function of salt concentration since their mean value is 1.98 ± 0.26 and are virtually identical to that obtained for the monovalent salts at 1 M concentration. In addition, the values of the conductance at a given surfactin concentration appears to be independent of the $CaCl_2$ concentration.

Finally, in order to shed some light on the influence of the membrane composition and structure on the observed macroscopic conductance, we also measured

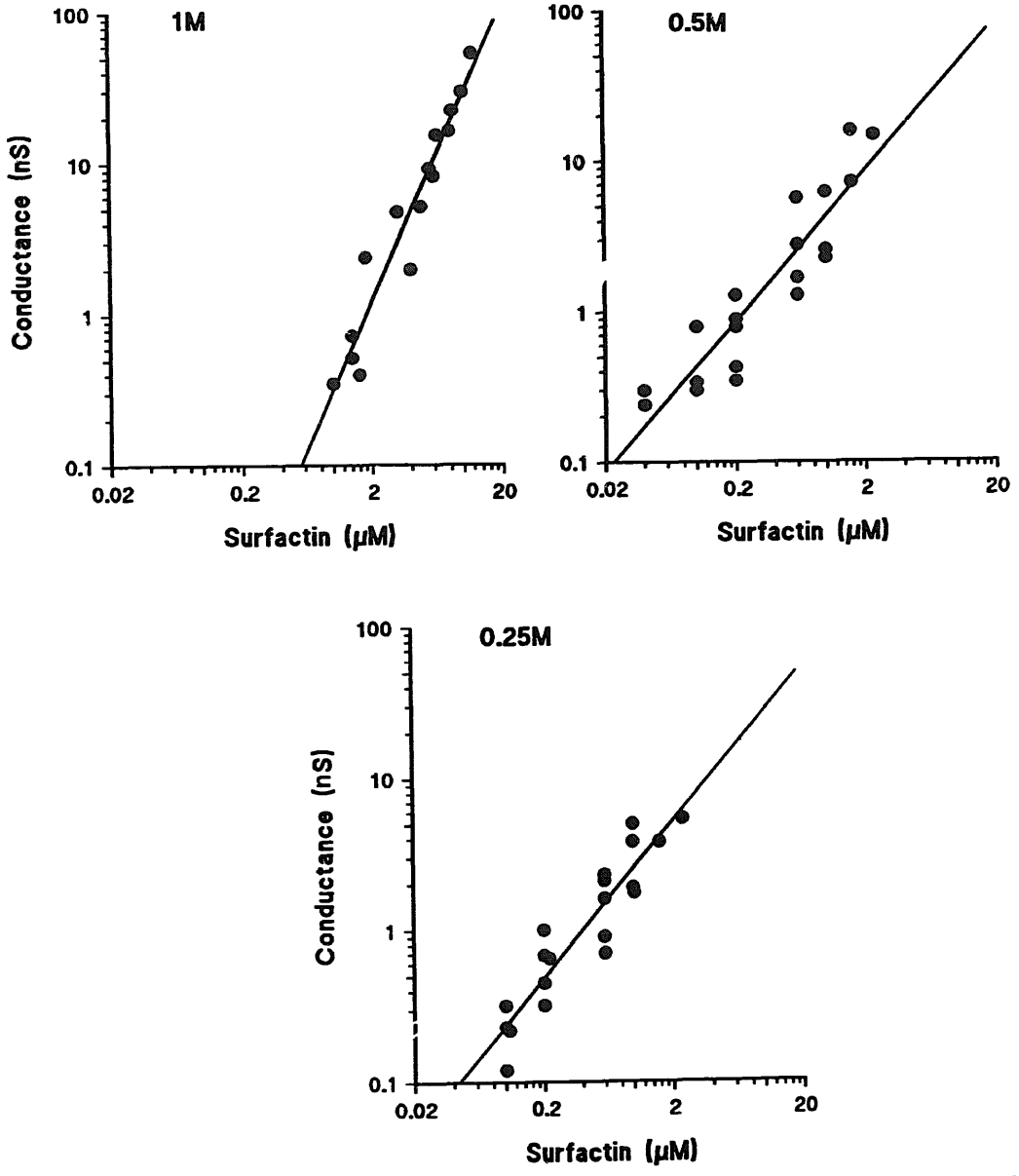

Fig. 9. Macroscopic conductance G_0 as a function of surfactin concentration for different concentrations of NaCl.

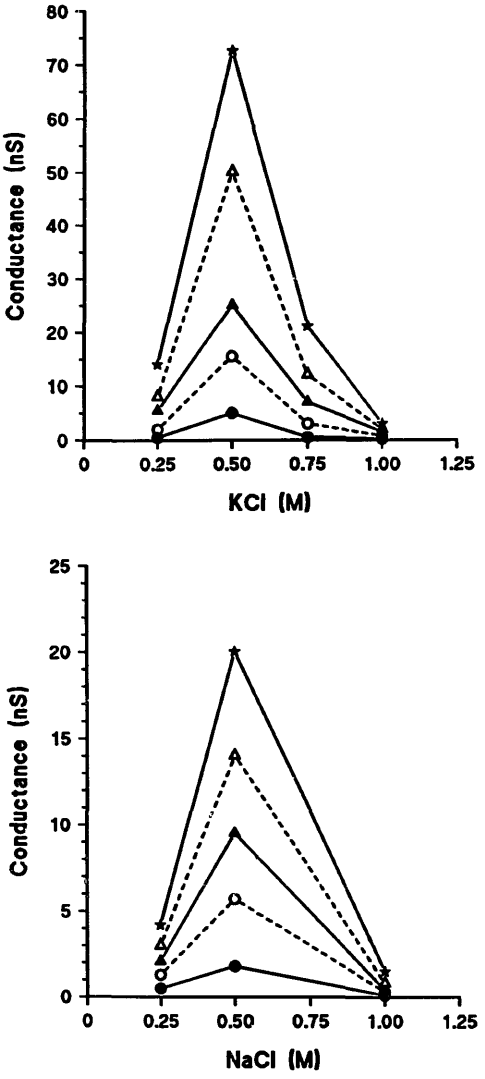

Fig. 10. Macroscopic conductance G_0 as a function of KCl and NaCl concentration for five different concentrations of surfactin. These plots are directly derived from those in Figs. 8 and 9, each point being the average of results obtained at 0.2 μM , 0.6 μM , 1.0 μM , 1.4 μM and 2 μM of surfactin.

the conductance of membranes made with glyceryl monooleate in squalene which are known to significantly increase carrier-mediated ion conductance [19,20]. As can be seen in Fig. 13, a similar behavior of the conductance as a function of surfactin concentration is obtained but at a surfactin concentration much smaller (10-fold) than that observed with a decane membrane. The values of the slopes obtained from the linear regressions are lower than those obtained with the decane membranes (1.45 and 1.59 for KCl and NaCl, respectively). However, note that the range of surfactin concentration over which the experiments could be conducted without the membranes breaking was smaller than with the glyceryl monooleate-decane experiments which could explain these somewhat lower values.

Discussion

Although many of its characteristics are very similar to those of iturin group antibiotics, which are also produced by several strains of *B. subtilis*, surfactin distinguishes itself by the fact that it does not contain any tyrosine residue and bears two negative charges. As it has been shown that methylation of the tyrosine residue changes importantly the characteristics of the pore-forming properties of iturin A [14], it was thus of great interest to study the conductance induced by surfactin in planar lipid bilayers.

As with iturin A, surfactin was found to induce current steps indicative of conductive pores through the lipid bilayer membrane. Also like iturin A, the conductance steps induced by surfactin covered a fairly wide range, none of the steps being clearly a multiple of a basic one. In addition, the increase in macroscopic conductance as a function of iturin A concentration gave, similarly to surfactin, a slope close to 2 on a log-log plot [14]. However, pores formed by surfactin also exhibited significant differences to those reported for iturin A. In similar glyceryl monooleate-decane bilayers, the conductance steps for iturin A in 1 M KCl ranged from 6 to 30 pS [13] while they ranged from 10 to 140 pS for surfactin. In egg-phosphatidylcholine bilayers, channel conductance for iturin A ranged from 7 to 50 PS. In contrast with iturin A [14], no channel activity could be detected whatever the surfactin concentration when it was dissolved directly in the membrane-forming lipid solution, suggesting an interaction of surfactin with the ions in the aqueous phase before its partitioning into the membrane. In addition, while for iturin A closing of channels appeared as frequent as openings, surfactin induced few channel closings. Also,

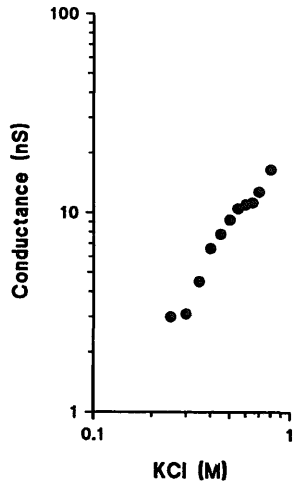

Fig. 11. Long term (> 20 min) values of the macroscopic conductance G_0 induced in a membrane already equilibrated with 0.8 μM aqueous surfactin in 0.25 M KCl to which different amounts of 3 M KCl are added to yield the indicated KCl concentrations (average of 2–5 membranes).

the amplitudes of the current fluctuations observed with iturin A increased with time, while the conductance steps induced by surfactin seemed time-independent. Finally, the single-channel selectivity found for surfactin with the various chlorides would suggest a cationic selectivity while only a small anionic selectivity was found for iturin A [13].

Histograms of conductance steps in KCl at different salt concentrations (Fig. 2) show a very large channel

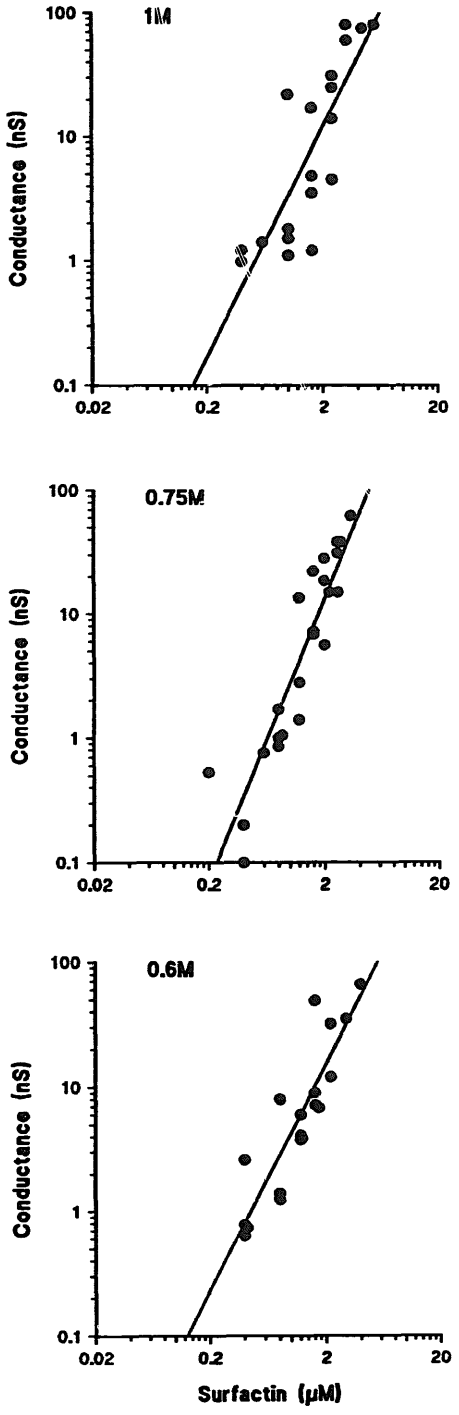

Fig. 12. Macroscopic conductance G_0 as a function of surfactin concentration for different concentration of $CaCl_2$ solution. As in the case of monovalent salts, slopes of straight lines are close to two.

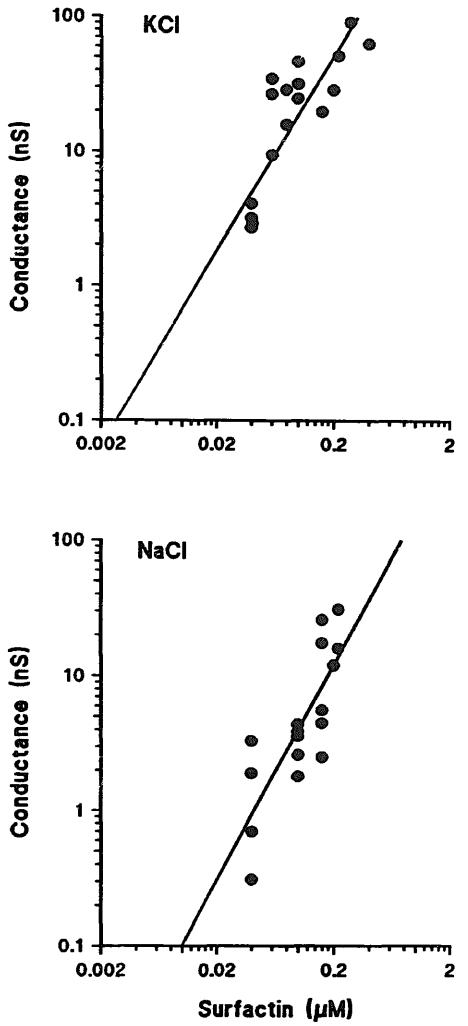

Fig. 13. Macroscopic conductance as a function of surfactin concentration for a membrane made with glyceryl monooleate in squalene for 1 M KCl and NaCl solutions.

diversity which reflects a complex behavior. Contrary to some other bacterial peptides, like the well-known gramicidin, which exhibits a pore-forming process characterized by a sharp maximum in the conductance histogram, surfactin induces channel values that cannot be interpreted as a multiple of a basic one. However, an analysis of these single-channels observed at different KCl concentrations, reveals that the single-channels that occur most frequently are also those that stay open for the longest period in each experiment (Fig. 2).

In addition, the smallest conductance steps also are the shortest in duration. Therefore, if the channel distribution remains the same, as more molecules partition in the membrane and more channels are formed, the macroscopic conductance should reflect the distribution of events shown in Fig. 2 and consequently the most frequent channels (which also show the longest open time). From the data shown in Fig. 2, one can calculate that from 29.8% to 78.1% of the macroscopic conduc-

tance is represented by the most frequent channel. This proportion increases to 56.0% to 89.7% when we consider the next lowest and highest conductance steps. The case is clearly demonstrated in Fig. 5 where the macroscopic conductance as a function of salt concentration obtained by addition of KCl salt to a membrane with an already established surfactin concentration (initial increase), parallels very well the most frequent channel conductance.

The above behavior contrasts strikingly with that of Fig. 10 for G_0 data obtained in different salt concentrations and where surfactin was increased stepwise. Indeed, Fig. 10 shows clearly that 0.5 M is a crucial salt concentration for surfactin incorporation into the membrane. Indeed, we see that the slope which is greater than 2 on a log-log plot from 0.25 to 0.5 M, changes abruptly at this particular concentration and becomes negative and as large towards 1.0 M. Interestingly, the behaviour as a function of ion concentration is identical whatever the surfactin concentration (and conductance level), supporting the notion introduced above that the interactions between surfactin, ions and membrane are independent of the absolute level of surfactin concentration in the aqueous phase and in the membrane. On the other hand, this particular behaviour of G_0 as a function of salt concentration, observed when surfactin is added to an aqueous solution already containing a given salt concentration (0.25–0.95 M), together with the fact that no channel could ever be recorded when surfactin was added via the lipid-forming solution, would strongly suggest an interaction of surfactin with the ions in the aqueous phase prior to partitioning into the membrane and forming conducting channels.

At this point, it would be appropriate to discuss the different steps that can be postulated to lead to the formation of channels in the membrane and consequently to the establishment of the membrane conductance. First, as mentioned above, the lipopeptide molecules most certainly interact with the ions in the aqueous phase. Indeed, due to the aspartate and glutamate residues, surfactin bears two negative charges at the pH at which the experiments were conducted (approx. 6.5, unbuffered solutions) and association of surfactin with cations is likely in order to neutralize the charge and allow the molecule to partition into the membrane. Germaine to this is the observation reported above that no channel could ever be seen when surfactin was introduced via the lipid-forming solution, and therefore prevented from interacting with the aqueous environment. Interestingly, in addition to interacting with ions, surfactin molecules may also associate in the aqueous phase to form oligomers or complexes of lipopeptides and ions. Indeed, it should be pointed out that at a pH of 6.5, simple fatty acids exhibit an appreciable amount of dimerization and in particular they form acid soaps of the type $(RCOO)_2HNa$ (e.g. in Na salts) which are overall electrically neutral [21]. Thus it is possible that a dimer of two surfactin molecules may be stabilized by two acid soap type bonds. Association of surfactin molecules whether in the aqueous phase or in the membrane is mandatory since a single lipopeptide molecule is too small to form a channel and at least two and more likely several molecules will need to be associated within the membrane to form a conducting pore. Therefore association of the surfactin molecules dimers or higher oligomers may take place solely in the aqueous phase and these preformed pores may then partition into the membrane. On the other hand, association may occur only within the membrane after the individual surfactin molecules have partitioned into the membrane. Alternatively, and quite likely, both of these mechanisms may take place sequentially, some association occurring in the aqueous phase followed by further association of the oligomers within the membrane. Indeed, the putative surfactin dimers or oligomers referred to above, which are expected to be labile in the aqueous phase, should be stabilized once inserted in the membrane. As can be seen in the Fig. 11, the steady-state conductance obtained after additions of KCl to a membrane with an already established surfactin concentration increases steeply with ion concentration. This would suggest that the partitioned surfactin molecules or oligomers, once in the membrane, remain there, thereby allowing further association as the ion concentration is increased, resulting in the observed increase in the number of conducting pores. In this context, the slope between 2 and 3 seen in Fig. 11 might correspond to a slope of 2 as a function of ion concentration for channel formation on which would be superimposed the ionic concentration dependence of the formed channel (Fig. 5). On the other hand, the observation of a slope of two as a function of surfactin concentration at 1 M salt concentration would suggest that two oligomers would be involved in the formation of a conducting channel.

With $CaCl_2$, we also found a slope of 2 for G_0 as a function of surfactin concentration, suggesting that with this divalent cation also, two surfactin oligomers are involved in forming a conducting channel. However, our data show that the macroscopic conductance G_0 does not vary significantly with Ca^{2+} concentration although the range of concentration studied is more limited (0.6–1.0 M as compared to 0.25–1.0 M). This would suggest that with the calcium ion, both the single-channel conductance and the number of conducting pores have reached a plateau in the range of concentration studied. The difference in behaviour in the presence of the calcium ion when compared to the monovalent ions is reminiscent of the behaviour of the salts of fatty acids (i.e. soaps). The monovalent salts are more water-soluble while calcium salts are more fat soluble and do not dissolve appreciably in water. Thus the systems containing calcium might be expected to

have a much different interaction with the lipid bilayer [22,23].

On the other hand, the decrease in G_0 which is seen in Fig. 10, when surfactin was added to a solution already containing salt at concentrations higher than 0.5 M, might be explained by a reduction in the aqueous concentration of partitionable lipopeptides-ions complexes or oligomers due to a relatively fast reaction in the aqueous phase and a decrease in the partition coefficient of these complexes between the aqueous phase and the membrane. Similarly, the possible different affinity of the lipopeptide molecules with the different ionic species, as well as the possible different partition coefficients of the various lipopeptide-ion complexes, might modulate their concentration in the membrane as we change the cation in the aqueous phase. This might eventually explain the different ionic selectivity patterns observed between single-channel events and macroscopic G_0 measurements (Fig. 4).

Finally, membranes formed from glyceryl monooleate in squalene (GMO-S) are considered relatively solvent-free and differ from the glyceryl monooleate-decane (GMO-D) membrane mostly by their thickness which is about half that of the latter (2.51 nm as compared to 4.95 nm) [19]. The major effect of this reduction in membrane thickness on carrier-mediated ion transport has been shown to be the increase by about a factor 10 in the rate of translocation of the mobile ion-carrier complex which was shown to be related to the reduction in the height of the electrostatic energy barrier across the membrane. In the present experiments, we have observed an increase by approximately a factor of ten in the macroscopic low-voltage conductance G_0 from GMO-D to GMO-S membranes. However, the amplitudes of the individual conductance steps, as well as their histogram obtained in GMO-S membrane were quite similar to those obtained with GMO-D membranes (data not shown). Therefore, the increase in G_0 observed with GMO-S membrane, which cannot be attributed to the aqueous phase, is probably related rather to an increased partition-coefficient of the surfactin-ion complexes across the membrane interface or to a larger extent of association of these complexes in the membrane which would lead to a larger number of conducting pores in the membrane.

In conclusion, we can state that although it does not contain any tyrosine, surfactin, a lipopeptide produced by certain strains of *B. subtilis*, induces voltage-independent and cation-selective channels in lipid bilayer membranes. Its behavior, as observed both in the macroscopic and the single-channel experiments, would suggest that the dimerization of two surfactin oligomers is involved in the pore-forming process that may occur sequentially in the aqueous and the lipid phases.

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