FT-IR Study of the Nature of the Proton and Li^+ Motions in Gramicidin A and C

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Gramicidin A and C as well as their proton and Li^+ complexes were studied by FT-IR spectroscopy. With an increasing number of hydrated and protonated channels, infrared continuous absorption is observed. It proves large proton polarizability due to very fast collective fluctuations and shifts of the excess proton in hydrogen-bonded single file water chains in the gramicidin channels. The infrared spectrum of the hydrated Li^+ cation in the channels shows a continuum in the far-infrared region. This continuum is explained in terms of Li^+ ion fluctuations and shifts between the electron lone pairs of two water molecules and at least three carbonyl O atoms of the backbone. The continuum indicates large Li^+ polarizability of these channels. If the Li^+-bonded system is polarized by an external electrical field, the diffusion of the Li^+−water complex in field direction is favored.

1. Introduction

Gramicidin synthesized by Bacillus brevis is widely used as an antibiotic agent. It is reactive to various gram positive organisms, gram negative cocci, and a number of fungi.[1,2] Gramicidin A (gA) and C (gC) are pentadecapeptides that form cation conductive transmembrane channels which are selective by transporting H^+ and Li^+ as well as other monovalent cations such as Na^+ and K^+.[3−5] Cell death can be caused by changes in cation concentrations, mainly by the loss of potassium ions.[6]

Understanding of ion translocation via ion channels through gramicidin is of utmost biophysical and medical relevance, because it leads to a deeper insight into the mechanism of ion conduction in membrane proteins in general. By understanding this mechanism, drug design of antibiotics based on the same principle could be enabled. Thus, gramicidin is one of the most popular model substances for studying the mechanism of ion translocation via ion channels through cell membranes.

Gramicidin A has the sequence HCO-$_L$Val^1-Gly^2-$_L$Ala^3-$_D$Leu^4-$_L$Ala^5-$_D$Val^6-$_D$Val^7-$_D$Val^8-$_L$Trp^9-$_D$Leu^{10}-$_L$Trp^{11}-$_D$Leu^{12}-$_L$Trp^{13}-$_D$Leu^{14}-$_L$Trp^{15}-NHCH$_2$CH$_2$OH.[7] In gramicidin gC the $_L$Trp^{11} residue is substituted by an $_L$Phe^{11} residue. gA can exist in various conformations depending on its environment.[8] These publications and other studies[9−12] have shown that standard gA channels in membranes are present as right-handed $\beta^{6.3}$-helical monomers.

Killian et al.[8] have found that it is possible to incorporate gA in the $\beta^{6.3}$-helical structure into membranes only from trifluoroethanol solutions. Recently, Luo and Baldwin[13] confirmed this result. In trifluoroethanol, however, gA is present in various rapidly interconverting conformers of left-handed and right-handed helices.[9]

X-ray diffraction studies have shown three types of double-helical structures of gA, i.e., antiparallel $\beta^{5.6}$-helices[14,15] and antiparallel $\beta^{6.4}$-helices, as well as a right-handed $\beta^{7.2}$-helix for the gA−CsCl complex.[16] All these X-ray results demonstrate that the transition from the solution into the solid state generates also these double-stranded helices. The knowledge of this structure is the basis of our FT-IR studies of gA and gC.

Langs[14] found by an X-ray study with 0.85 Å resolution an average inner channel diameter of 4.80 Å. This channel diameter varies from a minimum of 3.85 Å to a maximum of 5.47 Å. Simulation studies give a diameter of 4 Å and a length of the channel of 26 Å.[17]

In the past 40 years we have studied hydrogen bonds and hydrogen-bonded chains by infrared spectroscopy.[18−20] In the case of hydrogen bonds in which a double-minimum proton potential is present, or in the case of hydrogen-bonded chains with multiminimum proton potential, continuous absorptions have been observed in the infrared spectra. These continua demonstrate that the systems show so-called proton polarizabilities due to the fluctuations and shifts of the protons. These proton polarizabilities are about 2 orders of magnitude larger than usual polarizabilities due to the deformation of electron systems. Hydrogen-bonded chains with large proton polarizabilities are very suitable proton pathways. As we have shown, in the L_{550} intermediate of bacteriorhodopsin,[21] in the F_0 subunit of the ATP synthase,[22] as well as in corresponding models,[23] such hydrogen-bonded chains are present.

Very high polarizabilities are, however, caused not only by proton fluctuation and shifts but also by fluctuations and shifts of Li^+ in Li^+ bonds and by Na^+ in Na^+ bonds, respectively, as indicated by corresponding far-infrared continua (see ref 20).

In the following we studied the gramidicin A and C channels by FT-IR spectroscopy with regard to large H^+ and Li^+ polarizabilities and the meaning of these physicochemical principles for cation transport through cell membranes.

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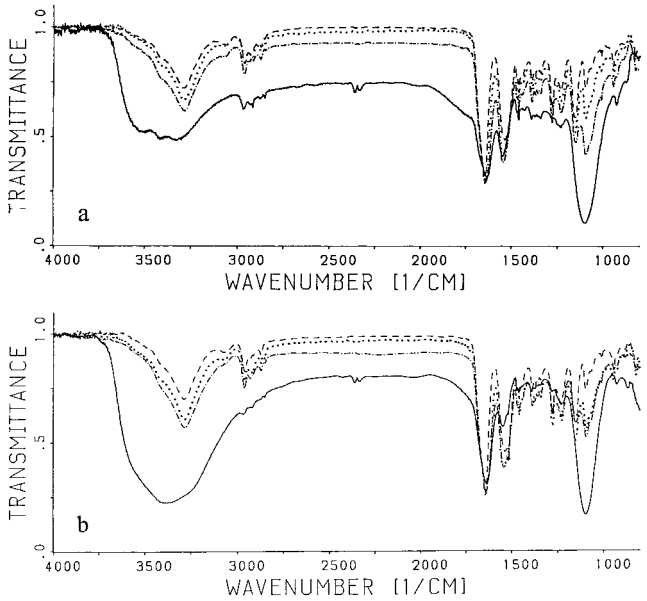


Figure 1. 1. ATR FT-IR spectra of (- - -) gramicidin, (• • •) gramicidin + 10 μL standard solution, (— — —) gramicidin + 30 μL standard solution, and (—) gramicidin + 100 μL standard solution: (a) gramicidin A, (b) gramicidin C.

2. Experimental Section

Gramicidin A and C are commercial products of Fluka and are used without any purification. Trifluoroethanol (spectroscopic degree) is purchased from Aldrich and distilled before use.

Preparation of the Samples. Two milligrams of gA or gC, about 6×10^{-7} mol, was dissolved in 1 dm^{-3} trifluoroethanol. To this solution were added 10 μL (1 proton per 10 channels), 30 μL (3 protons per 10 channels), or 100 μL (1 proton per channel) of the solution (standard solution): 0.005 mol dm^{-3} $HClO_4$ and 0.30 mol dm^{-3} H_2O in trifluoroethanol.

The Li^+ complexes were prepared by adding 2 mg of gramicidin in 1 dm^{-3} trifluoroethanol to a 30 μL (1 Li^+ per channel) solution of 0.042 mol dm^{-3} $LiClO_4$ in 0.30 mol dm^{-3} H_2O in trifluoroethanol.

The solutions of the gramicidin complexes with $HClO_4$ were spread on an internal reflection element and dried under a stream of dry nitrogen.

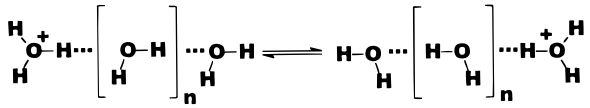
The measurements were performed in the spectrometer chamber flushed by dry air. The infrared spectra were recorded with a resolution of 2 cm^{-1}, using a Bruker IFS 113v FT-IR spectrometer equipped with an MCT detector. In the far-infrared a He-cooled bolometer was used. In the case of proton complexes, ATR spectra were taken with an internal reflection element. A germanium crystal 52 × 18 × 2 mm with an aperture angle of 45° was used. In the case of the Li^+ complexes, transmission spectra were taken from films prepared on a Si window.

3. Results and Discussion

Proton−Gramicidin Complex. The FT-IR spectra of films of proton−water −gramicidin complexes are shown in Figure 1a,b. In the series of spectra shown in Figure 1 as dotted, dashed−dotted, and solid lines an increasing amount of protons and water is present in the samples. The spectra of the films of pure gA and pure gC are also given as dashed lines.

In the infrared spectra of both gramicidins, continuous absorptions are observed with the addition of protons and water. These continua begin at about 3500 cm^{-1} and extend toward smaller wavenumbers. The dotted spectra show that continua are observed already in the 10 μL systems. The intensity of these continua increases strongly with the addition of further protons and water.

SCHEME 1



It is very important to notice that with the 10 and 30 μL samples no ν(OH) stretching vibration bands of water molecules are found in the region 3800−2800 cm^{-1}. The band at 3295 cm^{-1} is the amide A band, i.e., the stretching vibration band of the NH groups in the backbone.

Infrared continua demonstrate that hydrogen bonds or hydrogen-bonded systems with large proton polarizabilities are present. These polarizabilities are caused by very fast fluctuations of protons in hydrogen bonds or collective proton fluctuations of protons in hydrogen-bonded chains. The proton potentials in such hydrogen bonds with large proton polarizability are strongly influenced by their environment. This interaction is the reason for the occurrence of these continua.[18,19,24−28] Fluctuating potential barriers in the gramicidin channel have already been postulated by Läuger et al.[29] Infrared continua in the spectra of protonated and hydrated gramicidins prove that hydrogen-bonded chains with large proton polarizability are present in these systems (see Scheme 1).

Already a long time ago, Myers and Haydon[5] and Eisenmann et al.[30] have found that the single channel conductance of protonated gramicidin is more than 3 times larger compared with the conductance if other cations are present. Finkelstein and Anderson[31] discussed a Grotthus-like mechanism which is possible only if the water molecules form a continuous phase inside the channel. Later, Akeson and Deamer[32] discussed the proton conduction of the gramicidin channel. In the meantime it is known that eight water molecules are present in the channels in a single file arrangement.[33,34] These water molecules are bound with one OH group to the O atoms of the peptide groups, whereas the other OH group of these water molecules forms a hydrogen-bonded chain.

The binding of the water OH groups to the O atoms of the peptide bonds is proved by the amide bands in Figure 2. The amide I band [essentially the ν(C═O) vibration] shifts with the addition of water from 1640 to 1630 cm^{-1}, whereas the amide II band [vibration with large δ(NH) character] shifts from 1535 to 1550 cm^{-1}. These shifts are due to the binding of water OH groups to the carbonyl O atoms of the backbone via hydrogen bonds. Caused by this interaction with the water molecules, the hydrogen bonds in the backbone become stronger.

In the 100 μL sample all gramicidin channels are occupied by one proton and water molecules. In the spectrum of these samples (solid lines in Figure 1), intense continua are observed. Furthermore, three OH stretching vibration bands are found. In the case of gA (Figure 1a) these bands show pronounced maxima at about 3315, 3420, and 3540 cm^{-1}.

The intense continua prove that the hydrogen-bonded chains in the channels show large proton polarizability due to very fast proton fluctuations. The two water stretching vibration bands at 3315 and 3420 cm^{-1} show, however, that not all water molecules in the channels are involved in the fluctuation process. The band with maximum at 3315 cm^{-1} is caused by the OH groups of the water molecules bound to the O atoms of the backbone. The band at 3420 cm^{-1} is the stretching vibration of OH groups in water−water hydrogen bonds, not being

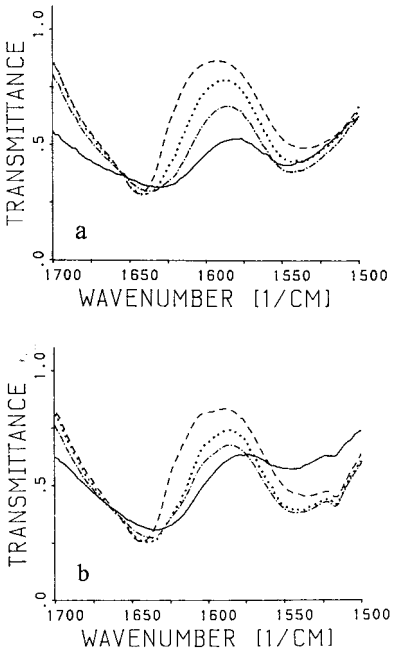


Figure 2. 2. ATR FT-IR spectra of (- - -) gramicidin, (· · ·) gramicidin + 10 μL standard solution, (— — —) gramicidin + 30 μL standard solution, and (—) gramicidin + 100 μL standard solution in the region 1700−1500 cm^{-1}: (a) gramicidin A, (b) gramicidin C.

involved in the charge fluctuation process. The band at 3540 cm^{-1} is probably caused by water molecules which are not involved in the water channel. These molecules are probably weakly bound to the backbone. These assignments of the water OH stretching vibrations have to be confirmed in the future by further studies of hydrated gramicidin as well as studies with model compounds.

An external electrical field can easily polarize the hydrogen-bonded chain with large proton polarizability. In this way the shift of the excess proton in field direction is favored. Thus, the large proton polarizability is the ultimate reason for the anomalously large proton conductivity of the channels with excess proton.

Let us compare gA and gC (Figure 1a,b). Up to 30 μL the spectra of proton−water−gramicidin complexes are comparable. The spectra with the 100 μL samples are, however, different. The intensity of the continuum with gA is higher than with gC, indicating that the proton polarizability of the hydrogen-bonded chains with the excess proton is higher with gA than with gC. On the other hand, the ν(OH) stretching vibration bands of the water molecules in the region 3700−3000 cm^{-1} are much more intense with gC compared to gA.

Instead of the $_{L}$Tryp^{11} residue in gA, a $_{L}$Phe^{11} residue is present in gC (see above). It is well-known that this substitution destabilizes the gC structure.[35] Thus, the continuum is less intense in gC compared to gA, since the hydrogen-bonded chains in which the charge fluctuates are destabilized in gC.

In gC a weak band is found at 1513 cm^{-1} which is not observed in the spectrum of gA, as shown by the comparison of parts a and b of Figure 2. This band is caused by the ν(C═C) ring stretching vibration of the phenylalanine residues which are only present in gC.

The Li$^+$ Channel. In Figure 3 the FT-IR spectrum of the gA−Li$^+$−water complex is shown. In this spectrum the broad band of the H_2O torsion vibration is observed with a maximum at about 500 cm^{-1}. With pure water this band is observed at about 700 cm^{-1}.[36] Hence, with the water in the channel this band is strongly shifted toward smaller wavenumbers. At the

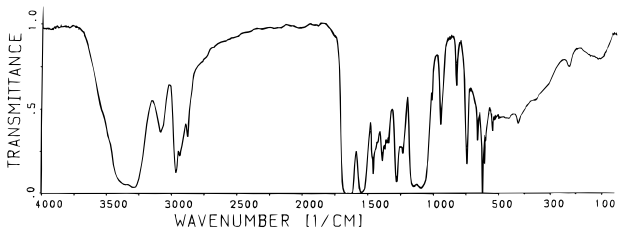


Figure 3. 3. FT-IR transmittance spectrum of the Li$^+$ complex of gramicidin A in the region 4000−50 cm^{-1}.

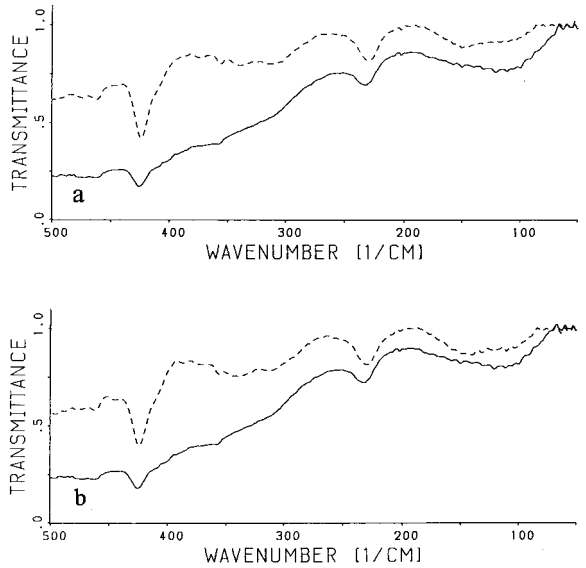


Figure 4. 4. FT-IR transmittance spectra of (- - -) gramicidins and their Li$^+$ complexes in the region 500−50 cm^{-1}: (a) gramicidin A, (b) gramicidin C.

low wavenumber slope of this band far-infrared continua begin at about 400 cm^{-1} and extend with decreasing intensity to about 50 cm^{-1}. This result is shown in Figure 4. From literature data it is well-known that Li$^+$ ions, which fluctuate in Li$^+$ bonds, cause the appearance of continua in the region below 400 cm^{-1} instead of the so-called Li$^+$ ion motion band usually observed at about 400 cm^{-1}.[20] This spectral feature is observed if one Li$^+$ ion fluctuates or if there is a collective motion of two or four Li$^+$ ions.[37] However, large Li$^+$ polarizabilities are also observed if one Li$^+$ ion fluctuates in a multiminimum potential. This result was obtained for Li$^+$ complexes of crown ethers.[38]

Thus, the continua in the IR spectra of Li$^+$ complexes demonstrate that the Li$^+$ ions fluctuate in the gramicidin channel in a double-minimum or multiminimum Li$^+$ potentials. Due to these Li$^+$ fluctuations such systems have large Li$^+$ polarizability.

Figure 5 shows the region of the stretching vibrations of the water molecules. Three bands are observed: one at about 3275 cm^{-1}, one at about 3400 cm^{-1}, and finally a weak shoulder at the high wavenumber slope of the latter band.

It is known from literature data that in the channels eight water molecules are present in a single file arrangement.[39−42] Two water molecules contact with their lone pairs the cations. With one OH group the water molecules are preferentially bound to the carbonyl O atoms of the peptide backbone, whereas with the other OH group these water molecules are cross-linked with each other in the single file arrangement. The shoulder at the high wavenumber slope is caused by free OH groups at the end of the water chains.[34,42−44] On the basis of these literature data, we assign the band at 3275 cm^{-1} to the water OH group bonded to the backbone, whereas the band at 3400 cm^{-1} is due

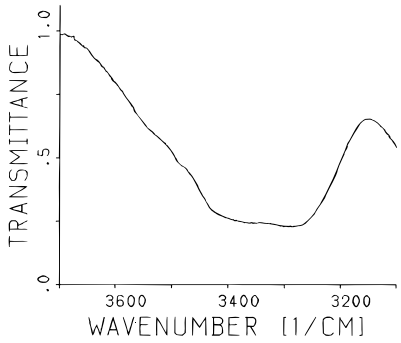


Figure 5. 5. FT-IR transmittance spectrum of the Li^+ complex of gramicidin A in the region 4000−3000 cm^{-1}.

to water OH groups in the single file arrangement. With regard to the band position (3275 cm^{-1}) the hydrogen bonds by which the water molecules are attached to the backbone are very strong. According to literature data, the dipole moments of the water molecules are increased.[34] This is in agreement with the formation of the very strong hydrogen bonds. The increase of the strength of the water hydrogen bonds by the interaction of their lone electron pairs with cations has already been studied a long time ago in detail.[36]

Now the question arises in which part of the gramicidin channel the fast fluctuation of the Li^+ cation takes place.

In each gramicidin molecule three pockets are present.[14,45] The cations migrate from pocket to pocket. The movement in the pore is strongly correlated, whereby the whole row of the ion and the water molecules is shifted.[34,43,46] In the case of Na^+ this shift, and especially the transition state, were studied by Roux and Karplus.[47] They found that the cations present in the wells interact directly with the lone electron pairs of two water molecules and with four O atoms of carbonyl groups. At the transition state the ion remains in close contact with two of the four carbonyl atoms. The whole column of cation and water molecules in the channel is shifted, and the file behaves like a single rigid body. These processes are relatively slow.[34,44,48] For this reason and with regard to the nature of these processes, they cannot be responsible for the occurrence of the continua. The reason for the infrared continua and the large Li^+ polarizability must be a much faster Li^+ fluctuation.

From crystal structure investigations[14] it is known that in the pockets of the channel the cross section contacts are 5.25 Å or greater. Roux and Karplus[47] assumed that the cations in the pockets interact with the lone pairs of two water molecules and with four O atoms of carbonyl groups of the backbone. Jordan[44] postulated that the cation in the pocket shuttles among the acceptors. Oscillation of the Li^+ ion on its binding positions was also found by Skerra and Brickmann.[43]

Hence, the Li^+ ions fluctuate in the pockets of the gramicidin channel in multminima Li^+ potentials. Furthermore, this Li^+ potential is modulated by the C═O librational motion. It was postulated by Roux and Karplus[49] that this librational motion should be found in the region 175−75 cm^{-1}. Indeed, in our far-infrared spectra in Figure 4 this band is observed in this region.

Due to the fluctuation and shifts of the Li^+ ions in such Li^+ potentials the Li^+ ions in the pockets cause large Li^+ polarizability, resulting in the continua observed in the far-infrared region. Conversely, these continua demonstrate large Li^+ polarizabilities. They are particularly large since the Li^+ ions are stronger bound to the acceptors compared with other alkali cations. The stronger binding results in higher barriers in the potential and, hence, larger Li^+ polarizabilities.

If an external electrical field is present due to the large Li^+ polarizabilities, the Li^+ ion is slightly shifted in field direction in the multiminimum potential. In this way the structure diffusion of the Li^+−water complex to the next pocket is favored.

Hence, the mechanism of the Li^+ ion shift in the channel is similar to that of the excess proton in aqueous acid solutions.[18] In these solutions the fluctuation of the excess proton in the hydrogen bond with proton polarizability in $H_5O_2^+$ is very fast, whereas the structure diffusion of $H_5O_2^+$ in the hydrate structure network is a much slower process.

4. Conclusions

The proton channels in gA and gC are proton pathways showing large proton polarizability. These channels are built up by water molecules, preferentially bound with one OH group to the carbonyl O atoms of the peptide groups. The other OH group of these water molecules makes up the proton pathway with large proton polarizability. These polarizabilities arise due to very fast collective fluctuations of the protons in multiminimum proton potentials of the hydrogen-bonded chain.

The Li^+ channels in gramicidins show large Li^+ polarizability due to very fast Li^+ ion fluctuations. With the Li^+ shift from one pocket of the channel to another not only the Li^+ ions but also the eight water molecules in the channel are shifted. This process is relatively slow compared to the fluctuation of cations in double-minimum or multiminimum potentials, respectively. Hence, these shifts of the Li^+ ions cannot be responsible for the Li^+ polarizability of the gramicidin channels. In the pockets the Li^+ ions fluctuate between the lone electron pairs of two water molecules, as well as three to four O atoms of the carbonyl groups. The fluctuation in these five to six minima Li^+ potentials results in the Li^+ polarizability indicated by the far-infrared continua. If these Li^+-bonded systems are polarized by an external electrical field, the Li^+ ion in this multiminimum potential is slightly shifted in field direction, favoring the structure diffusion of the Li^+−water complex to the next pocket of the gramicidin channel.

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