Molecular dynamics computations and solid state nuclear magnetic resonance of the gramicidin cation channel

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ABSTRACT This paper reports on a coupled approach to determining the structure of the gramicidin A ion channel, utilizing solid state nuclear magnetic resonance (NMR) of isotopically labeled gramicidin channels aligned parallel to the magnetic field direction, and molecular dynamics (MD). MD computations using an idealized right-handed β-heilx as a starting point produce a refined molecular structure that is in excellent agreement with atomic resolution solid state NMR data. The data provided by NMR and MD are complementary to each other. When applied in a coordinated manner they provide a powerful approach to structure determination in molecular systems not readily amenable to x-ray diffraction.

INTRODUCTION

Gramicidin A, a peptide of 15 amino acids, is a model for proteinaceous ion channels, for protein-lipid interactions, and for developing computational and experimental protocols for achieving high-resolution structures (1–3). To understand the mechanism of conduction in gramicidin, it is important to determine the channel structure as completely as possible. For the channel form of the polypeptide gramicidin A, an atomic resolution structure has not yet been achieved (4). Significant knowledge of gramicidin channel structure has arisen from spectroscopic data providing evidence to discriminate among possible secondary structures shown by calculation and model building to be energetically reasonable (5, 6). Two x-ray structures of gramicidin are available; however, neither is the dominant conducting form ("channel" form) in membranes (7, 8). The channel is formed in the lipid membrane by a combination of hydrophobic and hydrophilic forces acting in close proximity. Thus, a crystal of the channel form of the molecule is not readily obtainable under any crystallizing conditions. On the other hand, the uniaxial alignment of the gramicidin molecules in the liquid crystalline membrane matrix is advantageous for the solid state nuclear magnetic resonance (NMR) technique, which requires anisotropic motion and which can take advantage of the angular dependence of nuclear spin interactions. Recent studies using NMR (9, 10) have verified an earlier hypothesis (5) that the gramicidin channel is a β-helix, but have shown that the pitch is right-handed in both a micellar (9) and bilayer (10) environment, rather than left-handed as proposed earlier (11). The right-handed β-helical structure is also inferred from circular dichroism and conductance studies involving enantiomeric gramicidin analogues (12). In the present study, experimental solid-state ^{15}N NMR data obtained from oriented bilayer-bound gramicidin is used to evaluate various structural models for the gramicidin channel.

Solid-state ^{15}N NMR is a technique which provides direct atomic-resolution structural information in the form of orientational constraints. For a given ^{15}N nucleus within a molecule, the observed ^{15}N chemical shift is governed by the following orientational dependence:

$$\sigma_{obs} = \sigma_{11} \cos^2 \alpha \times \sin^2 \beta + \sigma_{22} \sin^2 \alpha \times \cos^2 \beta + \sigma_{33} \cos^2 \beta, \quad (1)$$

where σ_{11}, σ_{22}, and σ_{33} are the principal components of the ^{15}N chemical shift anisotropy (CSA) tensor, and α and β are the Euler angles relating the principal axis system (PAS) of the ^{15}N CSA tensor to the laboratory frame. Thus, for a single orientation of the channel, a single chemical shift is observed. To interpret the observed ^{15}N chemical shift frequency in terms of specific bond orientations, it is necessary to know the magnitude of the ^{15}N CSA tensor elements as well as the orientation of these elements relative to a reference frame fixed in the molecule. The magnitudes are readily obtained from powder pattern spectra (10). The orientational information is provided by the recent determination of the position of the PAS of the ^{15}N CSA tensor relative to the peptide plane for the Ala$_3$ and Leu$_4$ sites (13) for the Gly$_2$ site (14), and for the Val$_1$ site (unpublished results). Because the ^{15}N CSA tensor orientations for the Ala$_3$ and Leu$_4$ sites were nearly

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identical, similar orientations were assumed for the Ala_5, Val_6 and Val_7 sites. Thus, for a gramicidin structural model positioned with the channel axis parallel to the magnetic field, the predicted chemical shift for a particular site is easily calculated from the coordinates of the model. This is achieved via transformation from the PAS system through the molecular frame into the laboratory frame of reference. These predicted chemical shifts are compared with the experimental chemical shifts previously obtained from oriented samples positioned in the magnet with the channel axis parallel to the field (10).

It is useful to consider the sensitivity of the observed ^{15}N chemical shift frequency to geometrical features of the channel structure such as the pitch and roll of individual peptide planes. Specifically, we define pitch as the angle between the C_α-C_α axis and the channel axis, and roll as the degree of rotation of a peptide plane about the C_α-C_α axis from the position where the channel axis lies within the plane. Because σ_{11} and σ_{33} both lie in the peptide plane for all but the Val_1 site, the observed ^{15}N chemical shift is in general highly sensitive to pitch. For example, a ±5 degrees change of pitch for a typical even numbered site in the channel results in a 25-ppm change in chemical shift (15). Therefore, this solid-state NMR approach provides a narrow range of possible pitch angles for individual peptide planes, which is useful in discriminating between various structural models. Similarly, roll of a peptide plane sweeps the observed chemical shift through a range bounded approximately by σ_{33} and σ_{22}, depending on the pitch. Because σ_{22} and σ_{11} are very close in value, the observed chemical shift is also quite sensitive to roll (for a complete discussion, see reference 10). It must be noted, however, that the interplay between pitch and roll results only in an allowed region of space within which the peptide plane may lie, not in a single unique orientation. Therefore, whereas this approach may be a valuable tool in evaluation of structural models, the ^{15}N chemical shift alone cannot determine a unique structure. However, when combined with other experimental data such as ^{15}N-^1H and ^{15}N-^{13}C dipolar interactions, a limited set of possible ϕ, ψ torsion angle pairs for a given site can be achieved (16).

Fig. 1 shows the observed ^{15}N chemical shifts for gramicidin aligned with its channel axis parallel with the magnetic field and predicted chemical shifts for three different backbone structures. One structure is that proposed by Arseniev et al. (9). The other two are mean structures produced by MD computations for this report beginning from idealized left- and right-handed structures. These computations include water molecules but do not explicitly include the lipid membrane. The effect of the lipid is approximated by artificial restraints on the polypeptide motions (13). The left-handed starting structure for the MD is one proposed by Koeppe and Kimura (14), whereas the right-handed starting structure is just the right-handed analog of the left-handed starting structure. Table 1 lists the predicted ^{15}N chemical shifts for the three model structures as well as for the idealized right-handed starting structure. The experimentally observed values are included for comparison, with all chemical shifts referenced to a saturated aqueous $^{15}NH_4NO_3$ solution. It is seen that the right-handed structures clearly fit the experimental data better than the left-handed structure. Further, the right-handed MD structures are substantially better fits to the experimental data than the Arseniev et al. (9) structure. Because the latter structure is not a regular helix, and because it is derived from a spherical micellar environment rather than a planar bilayer environment, there is some ambiguity as to precisely how to define the channel

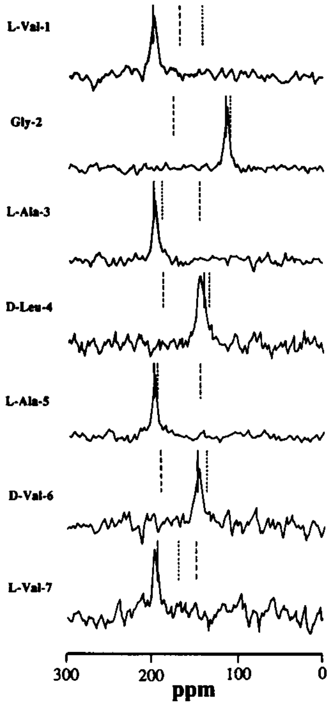
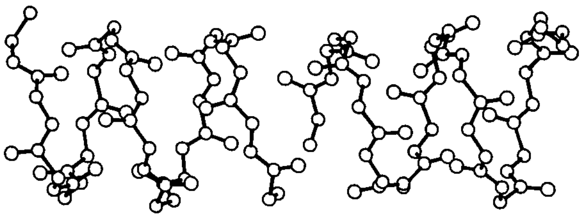


FIGURE 1 The observed ^{15}N chemical shift spectra for gramicidin aligned with its channel axis parallel to the magnetic field are shown. Vertical lines represent predicted positions of chemical shift resonances for three different structures of gramicidin. Solid lines are predictions of right-handed MD-produced structure starting with Koeppe-Kimura reference structure. Closely-spaced dotted lines are predictions of Arseniev et al. (9) right-handed structure. Widely-spaced dotted lines are predictions of left-handed MD-produced structure starting with Koeppe-Kimura reference structure. Differences between the right-handed structures are hypothesized to be primarily due to the presence of ions in the Arseniev et al. channels, in contrast to ion-free environment in the experiments and MD simulations reported here.

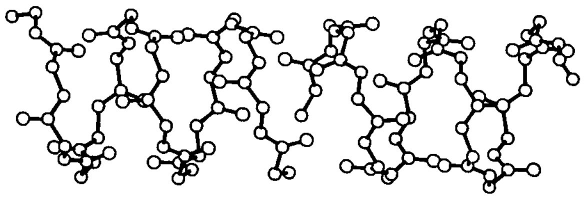
TABLE 1 Computed ^{15}N chemical shifts for structural models aligned with the channel axis parallel to the magnetic field direction

Site	MD RH* (ppm)	KK RH* (ppm)	Arseniev (ppm)	MD LH* (ppm)	Experimental (ppm)
Val$_1$	199	206	142	168	198
Gly$_2$	115	116	111	176	113
Ala$_3$	196	202	190	148	198
Leu$_4$	141	142	134	188	145
Ala$_5$	199	199	196	146	198
Val$_6$	146	143	137	190	145
Val$_7$	193	195	169	148	196

The experimental values are included for comparison. *MD RH = right-handed MD averaged, MD LH = left-handed MD averaged structure, KK RH = right-handed structure derived by the method of Koeppe and Kimura (18).



GRAMICIDIN - Arseniev Model



GRAMICIDIN - MD Averaged Model Structure

FIGURE 2 Side views of two right-handed backbone structures: (*a*) The Arseniev et al. (9) structure, and (*b*) the MD-produced right-handed structure starting with Koeppe-Kimura reference structure. Note that these structures are very similar to the eye, although they are clearly significantly different at the resolution of the NMR experiments. (Fig. 1).

axis orientation with respect to the magnetic field. We did the alignment by tilting the computed image of the molecule slightly, and then selecting the best fits to the experimental chemical shifts. The calculated shifts in Fig. 1 for the Arseniev et al. (9) structure represent this best effort to align the channel axis with the magnetic field.

The samples for the NMR spectra were prepared without the addition of permeant ions to the environment, and the computations were done for the situation with water but no ions in the channel. On the other hand, the Arseniev et al. (9) structure was derived from experiments with high concentrations of sodium chloride. Published computations (19–21) indicate that presence of ions in the channel effects a significant distortion of the backbone. Thus, it is inferred from Fig. 1 that the NMR chemical shift is sufficiently sensitive not only to distinguish the handedness of the peptide helix but also to detect distortion of the helix of the degree caused by ionic occupancy of the channel. This is a fairly subtle modification of the structure. To illustrate that point, side views of the Arseniev et al. (9) structure and the computed MD structure are shown in Fig. 2. To the eye, the differences are minor.

Because the MD computations produce a complete structure, we may use them to infer structural features beyond those shown directly by the NMR results to die. Whereas the NMR data is clearly inconsistent with the Arseniev et al. (9) structure, it is consistent with the idealized right-handed helix used as a starting structure. This match in chemical shift between observed and calculated is preserved in the right-handed MD structure, which is both functionally and energetically more reasonable. Of particular interest is the orientation of the carbonyl groups, because the carbonyl oxygens are likely to form numerous hydrogen bonds with water molecules in the channel, and electrostatic interactions with cations when they are present. The end view of the right-handed MD structure (Fig. 3) shows the tendency of the backbone to flex in such a way as to move the carbonyl oxygens closer to the center of the long channel

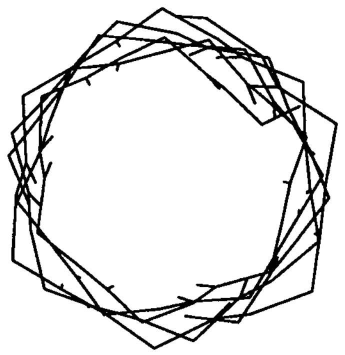


FIGURE 3 View looking down the channel axis of the backbone of the MD-produced right-handed structure. In addition to the peptide bonds, the carbonyl bonds are also shown explicitly. It is seen that there is a systematic tendency for the backbone to flex and the carbonyl bonds to tilt so as to move the carbonyl oxygens closer to the center of the channel, electrically polarizing the backbone in the radial direction. This prediction of the molecular dynamics is beyond the level of detail yet achieved by the NMR measurements, and may partially explain the preference of the channel for cations over anions (23).

axis, which we had previously seen in a computed left-handed structure (13). Whereas relieving the energetic strain associated with the starting structure, the molecular dynamics computations have succeeded in maintaining a remarkable degree of agreement with the chemical shift information. Furthermore, ^{15}N-^{1}H and ^{15}N-$^{13}C_1$ dipolar measurements on oriented samples confirm the chemical shift results and further restricts the conformational space for the nitrogen sites (16). The MD computations do not assume that the peptide bonds remain precisely planar, and indeed they do not. Fig. 4 shows the mean orientation relative to the long axis of the channel of each of the N-H bonds and C-O bonds in the right-handed structure. It is seen that the N-H and C-O orientations in each peptide bond differ by several degrees, so they are obviously not antiparallel to each other, as they would be in a planar peptide linkage. From a detailed analysis of both ^{15}N and carbonyl ^{13}C sites in the peptide linkage, the determination of the ω torsion angle about the peptide bond will unambiguously discriminate betweent these models. Because it is possible to get information directly from solid state NMR on the C-O orientations (22), it will be of interest to compare these predictions of MD to further data.

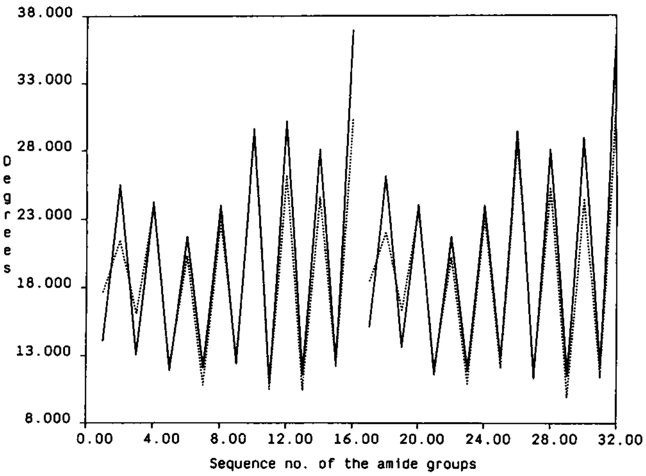


FIGURE 4 For the right-handed MD-produced structure, mean orientation of each of the N-H and C-O bonds in the channel backbone, expressed as the magnitude of the angle between the bond and the long axis of the channel. The solid line connects the values for the N-H bonds and the dotted line the values for the C-O bonds. For each monomer, the identifying number is lowest at the center of the channel and goes up as the mouth of the channel is approached. The alternating pattern arises from the fact that the basic repeating unit of the gramicidin helix is an L-D pair rather than a single amino acid. The slight asymmetry between the two monomers may be random or alternatively may be due to the asymmetry introduced by the water molecules in the channel lining up with their dipoles all pointing in the same direction along the channel axis. Note that there is systematically a difference of up to several degrees between the orientation of the N-H and C-O bonds in the same peptide bond. Therefore the N-H's and C-O's are not precisely antiparallel to each other as would be required in a rigid peptide plane.

These results show the power of solid-state NMR for structure determination in situations difficult for x-ray crystallography, and the complementarity between NMR and MD in making progress towards achieving a detailed refined structure. Where NMR and MD results are consistent with each other, the MD may be used to refine the structure beyond the precision of the NMR. Where the results are inconsistent with each other, they point up an area where our assumptions need to be reviewed. Through this interplay between MD and NMR we foresee solutions to long standing questions about structure and dynamics that will provide insights into the functioning of this channel at an atomic level. Already the question about the helix sense is resolved and the MD computations have brough about a remarkable coincidence between the model structure and the experimental data.

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REFERENCES

1. Hladky, S. B., and D. A. Haydon. 1984. Ion movements in gramicidin channels. *Curr. Top. Membr. Transp.* 21:327–363.
2. Jordan, P. C. 1988. Ion transport through transmembrane channels: Ab initio perspectives. *Curr. Top. Membr. Transp.* 33:91–111.
3. Pullman, A. 1987. Energy profiles in the gramicidin A channel. *Quart. Rev. Biophys.* 20:173–200.
4. Wallace, B. A. 1988. Gramicidin, a "simple" ion channel. *Curr. Top. Membr. Transp.* 33:35–50.
5. Urry, D. W. 1971. The gramicidin A transmembrane channel: A proposed π_{LD} helix. *Proc. Natl. Acad. Sci. USA.* 68:672–676.
6. Weinstein, S., B. A. Wallace, J. S. Morrow and W. R. Veatch. 1980. Conformation of the gramicidin channel: a ^{13}C nuclear magnetic resonance study of ^{13}C-enriched gramicidin in phosphatidylcholine vesicles. *J. Mol. Biol.* 143:1–19.

7. Langs, D. A. 1988. Three-dimensional structure at 0.86 Å of the uncomplexed form of the transmembrane ion channel peptide gramicidin A. *Science* (*Wash. DC*). 241:188–191.
8. Wallace, B. A., and K. Ravikumar. 1988. The gramicidin pore: crystal structure of a cesium complex. *Science* (*Wash. DC*). 241:182–187.
9. Arseniev, A. S., I. L. Barsukov, V. F. Bystrov, A. L. Lomize, and Yu. A. Ovchinnikov. 1985. ^{1}H-NMR study of gramicidin A transmembrane channel. Head-to-head right-handed, single-stranded helices. *FEBS* (*Fed. Eur. Biochem. Soc.*) *Lett.* 186:168–174.
10. Nicholson, L. K., and T. A. Cross. 1989. The gramicidin channel: an experimental determination of the right-handed helix sense and verification of β-type hydrogen bonding. *Biochemistry.* 28:9379–9385.
11. Urry, D. W., T. L. Trapane, and K. U. Prasad. 1983. Is the gramicidin A transmembrane channel single-stranded or double-stranded helix? *Science* (*Wash. DC*). 221:1064–1067.
12. Andersen, O. S., L. L. Providence, and R. E. Koeppe II. 1990. II. Gramicidin channels are right-handed β-helical dimers. *Biophys. J.* 57:100*a*. (Abstr.)
13. Teng, Q., and T. A. Cross. 1989. The in situ determination of the ^{15}N chemical-shift tensor in a polypeptide. *J. Mag. Res.* 85:439–447.
14. Teng, Q. Ph.D. thesis. 1990. Determination of nuclear spin tensor orientations and their application in the structure determination of gramicidin A by solid-state NMR. Florida State University, Tallahassee, Florida.
15. Nicholson, L. K., Q. Teng, and T. A. Cross. 1991. Solid-state nuclear magnetic resonance-derived model for dynamics in the polypeptide backbone of the gramicidin A channel by solid-state nuclear magnetic resonance. *J. Mol. Biol.* In press.
16. Teng, Q., L. K. Nicholson, and T. A. Cross. 1991. Experimental determination of the torsion angles in the polypeptide backbone of the gramicidin A channel by solid-state nuclear magnetic resonance. *J. Mol. Biol.* In press.
17. Chiu, S.-W., S. Subramaniam, E. Jakobsson, and J. A. McCammon. 1989. Water and polypeptide conformations in the gramicidin channel. A molecular dynamics study. *Biophys. J.* 56:253–261.
18. Koeppe, R. E., and M. Kimura. 1984. Computer building of β-helical polypeptide models. *Biopolymers.* 23:23–38.
19. Mackay, D. H. J., P. H. Berens, K. R. Wilson, and A. T. Hagler. 1984. Structure and dynamics of ion transport through gramicidin A. *Biophys. J.* 46:229–248.
20. Åqvist, J., and A. Warshel. 1989. Energetics of ion permeation through membrane channels. Solvation of Na^+ by gramicidin A. *Biophys. J.* 56:171–182.
21. Chiu, S.-W., and E. Jakobsson. 1990. Molecular dynamics of ion movement in gramicidin channels. *Biophys. J.* 57:98*a*. (Abstr.)
22. Smith, R., D. E. Thomas, F. Separovic, A. R. Atkins, and B. A. Cornell. 1989. Determination of the structure of a membrane-incorporated ion channel. Solid-state nuclear magnetic resonance studies of gramicidin A. *Biophys. J. 56:307–314.*
23. Sung, S.-S., and P. C. Jordan. 1987. Why is gramicidin valence selective? A theoretical study. *Biophys. J.* 51:661–672.