SOLID STATE NUCLEAR MAGNETIC RESONANCE APPROACH FOR DETERMINING THE STRUCTURE OF GRAMICIDIN A WITHOUT MODEL FITTING

T. A. CROSS
Department of Chemistry, Florida State University, Tallahassee, Florida 32306-3006

Recently, solid state nuclear magnetic resonance (NMR) methods have been used with magnetically aligned samples to determine bond orientations with respect to the magnetic field in the backbone of polypeptides (1, 2). From the bond orientations the orientation of the planar peptide linkages can be determined. In these initial efforts the presence of a distorted α helix was inferred from the peptide linkage orientations and model-fitting the data (2). However, it is possible to take advantage of the tetrahedral geometry of the α carbon joining adjacent peptide linkage planes and steric considerations to determine the three-dimensional structure without model-fitting.

It has been shown that the ion-conducting channel conformation of Gramicidin A in a phospholipid bilayer can be magnetically aligned (3). While a structural model of this channel (4) has withstood many challenges over the last 14 years, it remains a model for which there is very little direct structural data. The model does not explain physical data such as the sequence specificity of ion conductance (5) and the location of a specific site for ion binding in the channel (6). To illustrate the solid state NMR approach for determining the structure of Gramicidin a priori, the NMR data has been predicted for a tripeptide sequence, –D Val_8–L Trp_9–D Leu_{10}–, from the atomic coordinates of the structural model. The Val_8–Trp_9 peptide linkage will be referred to as linkage *A* and the Trp_9–Leu_{10} linkage as *B* (see Fig. 1).

The only NMR data used in this analysis are the N–H (N–H) and N–C_1 (N–C_1) dipolar interactions predicted for the Gramicidin channel aligned such that the channel axis is parallel with the magnetic field as shown by Van Echteld et al.. The predicted values of the dipolar couplings, $\Delta\nu$, were obtained from the following equation and the values are given in Table I.

$$\Delta\nu = \nu_{\parallel}(3\cos^2\theta - 1). \quad (1)$$

θ is the angle between the bond and the magnetic field. From the absolute value of $\Delta\nu$ the possible bond orientations are determined (Fig. 1). The determination of θ using this equation will result in an ambiguity in the sign of $\cos\theta$. Because the absolute value of $\Delta\nu$ is obtained from the NMR spectrometer, for values of $\Delta\nu \leq \nu_{\parallel}$ there are a total of four values of $\cos\theta$ obtained. When $\Delta\nu > \nu_{\parallel}$ there is only one possible sign for $\Delta\nu$ and therefore, two possible values for $\cos\theta$. A property of helical structures oriented approximately parallel with the field is that $\Delta\nu$ is generally $>\nu_{\parallel}$ for the N–H bonds. This is true for Gramicidin oriented in a lipid bilayer as shown in Table I (3). The two values for $\cos\theta_{N-H}$ of linkage *B* will lead to duplicate sets of torsion angles—in effect, one set where the molecule is oriented with respect to the positive field direction and one where the molecule is similarly oriented with respect to the "negative" field direction. This duplication is eliminated by considering only one of these θ_{N-H} angles. This is a one-time elimination, for all other dipolar couplings both values of θ will be considered.

For N–C_1 dipolar couplings, which are typically small when the N–H couplings are large, four values of θ are obtained. This leads to four possible combinations of N–H and N–C_1 orientations in linkage *B*. Two of these combinations are eliminated because they are inconsistent with the HNC_1 bond angle. The orientation of two bonds with respect to the magnetic field does not uniquely define the orientation of a plane with respect to the field. An ambiguity exists for the sign of the direction cosine relating the axis normal to the plane with the field. Therefore, for linkage *B*, four possible orientations need to be considered.

For these orientations a laboratory axis frame can be assigned (see Fig. 2) with the *Z* axis parallel with the field and the *X* and *Y* axes arbitrarily placed for an orthogonal axis system. By defining the orientation of this peptide

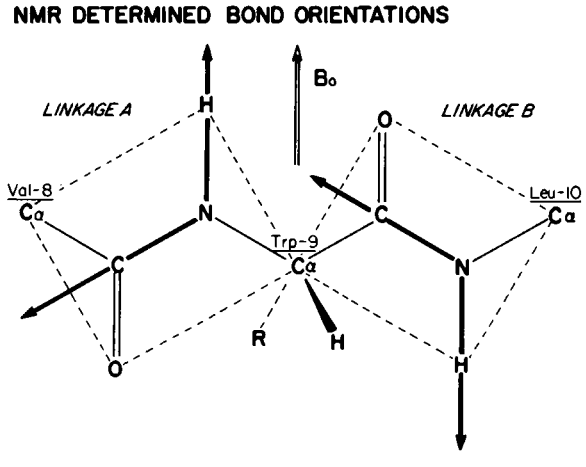

FIGURE 1 A tripeptide sequence in Gramicidin A illustrating the planar nature of the peptide linkages and the bonds in boldface for which the NMR data was predicted. The orientation of these vectors with respect to the field, B_0, is determined from the dipolar coupling between directly bonded atoms in an oriented sample. The carbonyl carbons are referred to as the C_1 atoms throughout this communication.

BIOPHYS. J. © Biophysical Society · 0006–3495/86/01/124/03 $1.00
Volume 49 January 1986 124–126

TABLE I

THE PREDICTED DIPOLAR COUPLINGS: $\Delta\nu$, FOR THE TWO PEPTIDE LINKAGES OF THE TRIPEPTIDE, Val – Trp – Leu, IN GRAMICIDIN

Dipolar Couplings	Val_8 – Trp_9 Linage A	Trp_9 – Leu_{10} Linkage B
*N—H	+15.30 kHz	+17.08 kHz
‡N—C_1	±1.03 kHz	±0.51 kHz

*ν_1 = +9.45 kHz (2).
‡ν_1 = −1.33 kHz (2).

linkage the locations of six atoms are determined, including the location of the Trp_9 and Leu_{10} α carbons. The NMR data for the adjacent peptide linkage, *A*, can be used to determine the possible orientations for the N–C_α bond of linkage *A*. Two possible orientations must be considered for the N–H bond and four orientations for the N–C_1 bond, resulting in eight possible combinations. Only four of these combinations allow for the known HNC_1 bond angle and therefore, four N-C_α bond orientations are to be considered with the four linkage *B* orientations. Of the sixteen combinations of N-C_α and N–C_1 orientations two are inconsistent with the $NC_\alpha C_1$ bond angle. Again the orientation of two bonds with respect to the field describes a plane for which the normal to the plane is defined relative to the field with an ambiguity in the sign of the direction cosine. Twenty-eight values of the ψ torsion angle result. Of these only nine fall in stable regions of the Ramachandran diagram. To determine the ϕ torsion angle the location of C_1 in linkage *A* must also be determined. The ambiguity in the sign of the direction cosine relating the axis of the normal to the plane of linkage *A* with the field doubles the number of coordinate sets. Of the 18 sets of direction cosines only 10 fall in sterically allowed regions of the Ramachandran diagram. These 10 possible relative orientations of the two peptide linkages are described by the torsion angles given in Table II. The ϕ angle of −144° and

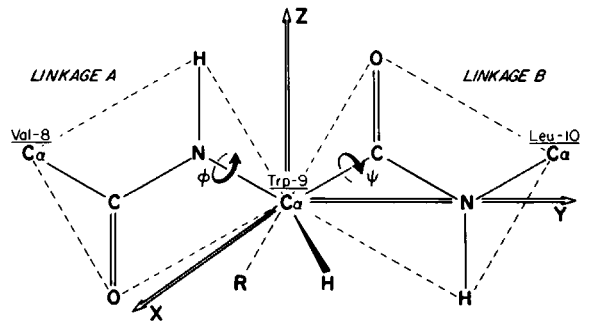

FIGURE 2 The same sequence as shown in Fig. 1, but here the orientation of the laboratory frame is shown. *Z* is parallel with the field and *X* and *Y* and placed in orthogonal relationships to form an axis system. This frame is first defined for the atoms of linkage *B* and then the bond orientations in linkage *A* are determined with respect to the lab frame. The ϕ and ψ torsion angles that characterize the relative orientation of the peptide linkage planes are also shown.

TABLE II

THE POSSIBLE RELATIVE ORIENTATIONS FOR THE TWO PEPTIDE LINKAGES OF THE TRIPEPTIDE, Val – Trp – Leu, IN GRAMICIDIN DETERMINED WITHOUT MODEL FITTING FROM PREDICTED NMR DATA

Conformation	ϕ	ψ
1	−142.8°	+132.7°
2	−142.8°	+98.7°
3	−129.6°	+101.8°
4	−128.9°	+163.9°
5	−128.9°	+142.6°
6	−122.9°	+112.8°
7	−122.6°	+132.7°
8	−122.6°	+98.7°
9	−109.4°	+101.8°
10	−102.7°	+112.8°

ψ angle of 132° correspond to the torsion angles of the model structure from which the NMR data was predicted.

From the dipolar NMR data for two bonds in a single peptide linkage it is conceivable that up to 16 sets of coordinates can be generated. For an adjacent peptide linkage up to 32 sets of coordinates are possible resulting in as many as 1,024 possible relative orientations of the peptide linkages. By taking into consideration the geometry of the atoms involved and steric hindrance, this number has been reduced to 10 possible solutions. These solutions all fall in the region of the β secondary structures, none of them is near either the collagen triple helix or α-helix regions of the Ramachandran diagram.

The number of possible solutions for the structure of Gramicidin, as it is further elaborated will decrease, as many of the solutions will not result in reasonable secondary structures. Furthermore, from these sets of orientations it is clear that as more NMR data is accumulated that the number of solutions can also be reduced. For instance, determining any of the C_α bond orientations directly will reduce the possible solutions by approximately a factor of two. Another attractive possibility is to interpret the C_1 chemical shift anisotropy, which has a high asymmetry coefficient and would therefore provide information about the orientation of the axis normal to the plane of the peptide linkage. For now, it is shown that the solid-state NMR approach for determining polypeptide backbone structures does not have to depend on model-fitting the observed data.

The author would like to thank Professor Dan Urry for providing the coordinates of the structural model of Gramicidin A.

Received for publication 11 May 1985.

REFERENCES

1. Cross, T. A., P. Tsang, and S. J. Opella. 1983. Comparison of protein and DNA backbone structures in fd and Pf1 bacteriophages. *Biochemistry*. 22:721–726.

2. Cross, T. A., and S. J. Opella. 1985. Protein structures by solid state NMR: residues 40–45 of fd coat protein. *J. Mol. Biol.* 182:367–381.
3. Van Echteld, C. J. A., B. de Kruijff, A. J. Verkleij, J. Leunissen-Bijvelt, and J. de Gier. 1982. Gramicidin induces the formation of non-bilayer structures in phosphatidylcholine dispersions in a fatty acid chain length dependent way. *Biochim. Biophys. Acta.* 692:126–138.
4. Urry, D. W. 1971. The Gramicidin A transmembrane channel: a proposed π(L,D) helix. *Proc. Natl. Acad. Sci. USA.* 68:672–676.
5. Bamberg, E., K. Noda, E. Gross, and P. Lauger. 1976. Single-channel parameters of Gramicidin A, B, and C. *Biochim. Biophys. Acta.* 419:223–228.
6. Urry, D. W., K. U. Prasad, and T. L. Trapane. 1982. Location of monovalent cation binding sites in the Gramicidin A channel. *Proc. Natl. Acad. Sci. USA.* 79:390–394.