Anisotropic Chemical Shift Perturbation Induced by Ions in Conducting Channels

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Anisotropic chemical shifts observed from solid-state NMR spectroscopy of uniformly aligned samples can be influenced by three primary factors: a change in orientation of the nuclear site, a change in dynamics, or a change in the chemical shift tensor element magnitudes or orientation to the molecular frame. These features are particularly attractive for characterizing the influence of ions in ion conducting channels. Cation binding results in far more subtle effects than had previously been imagined. Prior to the analysis of the first solid-state NMR characterizations of ion binding [1,2] the experimental data were primarily in the form of a few water-soluble protein structures in the Protein Data Bank to which monovalent ions were bound [e.g. 3,4]. Such binding sites showed optimized solvation for the ions associated with strong binding. Computational modeling efforts on ion channels, for the most part, also showed substantial structural deformation upon ion binding [5–7]. We now realize that much better models for how ions interact with channels can be realized from the characterization of substrate binding to enzymes, for which we have a great deal of information represented in every biochemistry textbook. A delicate balance of molecular interactions and thermodynamic parameters has evolved for enzymes, so that substrates are attracted to the active sites of proteins while not compromising the primary function of these proteins to conduct chemistry on the substrates and to release the products efficiently. Similarly, ions must be attracted to the channel and yet the primary function of these proteins is to facilitate the transfer of ions from one side of the membrane to the other.

Here, we describe how this can be done through a model system, the monovalent cation channel gramicidin A, produced by *Bacillus brevis*, to lyse cells in its environment and from the lysate the bacillus harvests amino acids following the additional export of proteases. This unique polypeptide has an alternating sequence of D and L amino acid residues that forms a β-strand with all of its side chains on one side of the strand resulting in a helical conformation with an aqueous pore approximately 4.5 Å in diameter. The high-resolution structure has been fully characterized using solid-state NMR orientational restraints from uniformly aligned samples in lipid bilayers (PDB # 1 mag) [8,9]. Analysis of the refined structure illustrates the unique high-resolution detail of this time averaged structure [10]. This polypeptide spans the lipid bilayer as a symmetric dimer [11], the amino-termini of which are formylated at the bilayer center. The approximate location of two symmetric ion binding sites in the vicinity of the monolayer interfacial region was initially characterized by X-ray diffraction [12]. Gramicidin shares a number of important features with the more recently characterized K^+ channels [13,14]. In particular, it is the polypeptide backbone and the carbonyl oxygens that provide much of the solvation environment following ion dehydration in the ion binding site of gramicidin A and in the selectivity filter of the KcsA channel. However, in KcsA the ion binding sites are considerably closer together (approximately 7 Å for KcsA [15] and approximately 20 Å for gramicidin A) permitting stronger ion binding and a much higher degree of ion selectivity. Nevertheless, the principles gleaned from studies of gramicidin appear to have very general applicability.

Solid-state NMR of uniformly aligned samples leads to high-resolution spectra. The frequencies from chemical shift, dipolar and quadrupolar interactions can be used as structural restraints by interpreting the frequencies within the context of the appropriate motionally averaged spin interaction tensor [16–18]. Here, we primarily describe the use of ^{15}N NMR spectroscopy of the amide nitrogen sites in the polypeptide backbone of gramicidin A in hydrated liquid crystalline preparations. These tensor element magnitudes in both static and liquid crystalline environments have been characterized for each of the amide sites [8,19] and many of the tensor orientations to the molecular frame have also been characterized [19,20]. These ^{15}N anisotropic chemical shifts represented some of the data used for solving the 3D structure in the absence of ions. The anisotropic shifts in the cation binding region change upon the introduction of ions [21,22] (Figure 1). The influence of the ions is surprisingly small compared to the 40–50 ppm anisotropic shifts calculated [23] from the structural changes predicted in the first molecular dynamics study of gramicidin ion binding [5]. Consequently, it

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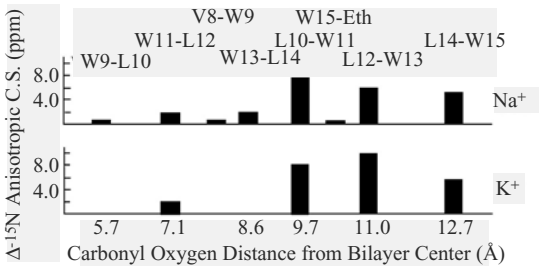

Fig. 1. Change in anisotropic chemical shift upon binding ions to the gramicidin channel (>80% double occupancy based on known binding constants [34, 35]) as a function of carbonyl oxygen distance from the bilayer center.

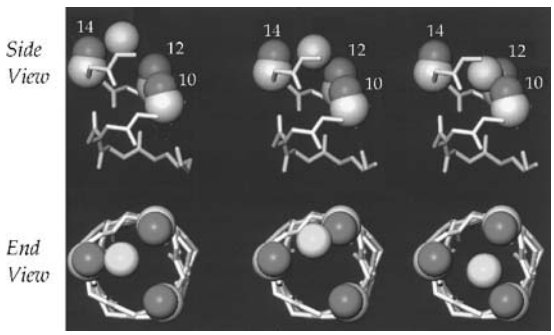

Fig. 2. Van der Waals surfaces are shown for the carbonyl groups of Leucine 10, 12, and 14, as well as for a K^+ ion interacting with these carbonyls. Ions are not able to interact with all three carbonyl oxygens at one time. (See also Plate 33 on page XXX in the Color Plate Section.)

appears as if the structural changes are likely to be small. In addition, the ion's influence is focused on three amide nitrogens that are in peptide planes having their carbonyl oxygens exposed at the end of the channel. If the cation interaction with the carbonyl oxygens was similar throughout the channel then the magnitude of the anisotropic chemical shift change would reflect the time the ions are positioned in the vicinity of each carbonyl oxygen and hence these sites with a significant change in anisotropic chemical shift reflect the cation binding sites.

This assumption is defendable, because we observe small anisotropic chemical shifts for sites throughout the gramicidin structure with a wide variety of ions. However, these chemical shift perturbations are all less than or equal to 2 ppm whether they are in the vicinity of the three exposed carbonyls or nearer the bilayer center. In other words, there is no rational to think that the cation binding site extends further toward the bilayer center than the Leu_{10} carbonyl (9.7 Å). Even the Trp_{15} carbonyl is unaffected despite its distance of 10.3 Å from the bilayer center. Indeed, for an optimal interaction between a cation and a carbonyl oxygen the ion should be on the axis or near to the axis of the dipolar vector. For the Leu_{10} carbonyls (and similarly for Leu_{12} and Leu_{14} carbonyls) with its dipole oriented towards the aqueous environment the ion would be positioned at least an angström further away from the bilayer center (10.7 Å). However, for the Trp_{15} carbonyl the dipole is oriented toward the hydrophobic interstices of the bilayer and this would suggest that for the ion to have a significant interaction with this site it would need to be at least another angström further into the channel (9.3 Å). This easily accounts for the strong chemical shift perturbation in the Leu_{10} carbonyl peptide plane and the very weak perturbation in the Trp_{15} carbonyl peptide plane.

Since the structure is known to high resolution and since the structure does not change substantially upon ion binding as indicated above, it can be preliminarily concluded that the ions are not interacting with all three of these carbonyl groups at the same time (Figure 2). In other words the carbonyl oxygens are not rotating in toward the channel axis and toward the cation to the extent necessary for the ion to be solvated by multiple carbonyls simultaneously. There are numerous important conclusions that follow from this unique solid-state NMR result, but first we should inspect some of our assumptions.

Upon binding monovalent ions the shift in the amide ^{15}N chemical shifts could be the result of a change in structure, dynamics, or tensor. By using three separate tensors in a single peptide plane and assuming simply a change in orientation of the peptide plane: data from the carbonyl carbon (^{13}C chemical shift) [1] suggest a change in orientation of 9°; the ^{15}N chemical shift, a change of 7° and the ^{15}N–^{2}H dipolar interaction, a change of 2° [24] (Figure 3). Such a discrepancy in the results suggests that the time averaged structural deformation

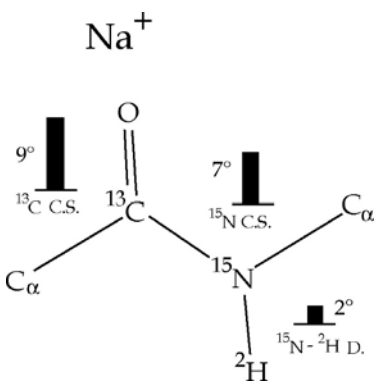

Fig. 3. Interpretation of the anisotropic spin interactions in the Leu_{12}-Trp_{13} peptide plane upon binding Na^+ (>80% double occupancy).