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The backbone ^{15}N chemical shift tensor of the gramicidin channel. A molecular dynamics and density functional study

Thomas B. Woolf [a], Vladimir G. Malkin [b], Olga L. Malkina [b], Dennis R. Salahub [b,c], Benoît Roux [a,b,c]

[a] *Groupe de Recherche en Transport Membranaire (GRTM), Université de Montréal, C.P. 6128, Succ. Centre-ville, Montréal, Québec, Canada H3C 3J7*

[b] *Département de Chimie, Université de Montréal, C.P. 6128, Succ. Centre-ville, Montréal, Québec, Canada H3C 3J7*

[c] *Centre de Recherche en Calcul Appliqué (CERCA), 5160 Boulevard Décarie, bureau 400, Montréal, Québec, Canada H3X 2H9*

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Abstract

The short-time-scale fluctuations in ^{15}N chemical shift tensors are examined for an ensemble of N-methylacetamide complexes constructed from a molecular dynamics (MD) trajectory of the gramicidin A channel in a fully hydrated phospholipid bilayer. Sum-over-states density functional perturbation theory (SOS-DFPT) calculations reveal fluctuations in the magnitudes and orientations of the individual tensor components on the picosecond to femtosecond time scale. The MD/SOS-DFPT technique emerges as a valuable tool for gaining insight into the nature of the terms that enter the motionally averaged spectra observed experimentally. More extensive sampling should provide first-principles predictions of the spectra under experimental conditions.

1. Introduction

Solid state NMR is a powerful approach to obtaining information about the conformation of membrane bound proteins which are otherwise very difficult to study experimentally [1,2]. In particular, measurements of the backbone ^{15}N chemical shift of a protein embedded in a bilayer can provide constraints on the average orientation of the peptide linkages which can be used to determine the three-dimensional structure [1]. One important assumption to interpret the experimental data in terms of a three-dimensional structure is that the ^{15}N shielding tensor determined in a powder sample can be transferred to the situation of a protein in a fluid bilayer environment. An underlying assumption is that the tensor orientation is fixed relative to the local molecular frame and that the component magnitudes are constant [1]. In other words, the experimental powder tensor components are attached to the molecular frame of the amide group, C–N–H, and the observed chemical shift results mainly from the re-orientation of the peptide linkages with respect to the magnetic field [1]. Evidently, such an approximation describes well the dominant contribution on the solid state NMR time scale although it is clear that the measured spectrum results from an average over rapid fluctuations, occuring on the femtosecond and picosecond time scale, in shielding tensor component magnitudes and directions. However, the importance of such fluctuations for a protein in a fluid phospholipid bilayer is not known.

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This Letter examines the nature of the rapid variations in shielding tensor component magnitudes and directions and the way they contribute to determining the observed spectrum for the case of a protein in a fluid phospholipid bilayer. In particular, we ask the question of whether the shielding tensor which was determined from the powder sample may be transferred rigidly the more rapid time domain relevant to molecular dynamics simulations. It may be expected that the variations in the local environment of the backbone and the fluctuations in its geometry, both necessarily present at room temperature [3–5], could influence the tensor orientation and magnitudes. For a meaningful study, it is thus necessary to consider an ensemble of configurations that is representative of the fluctuating protein in a membrane environment. In addition, the chemical shift tensor is a sensitive property of the electronic structure around the nucleus and its calculation requires high level electronic structure theory [6–11]. Due to recent advances in the molecular dynamics simulation of membrane proteins in an explicit phospholipid bilayer [4,5] and the development of the sum-over-states density functional perturbation theory (SOS-DFPT) [10,11] for calculations of the chemical shifts, it is now possible to address this problem at the microscopic level.

The goals of this Letter are (i) to explore the nature of the instantaneous microscopic fluctuations in the ^{15}N chemical shift tensor of a transmembrane protein and (ii) to demonstrate that the SOS-DFPT method for chemical shifts is applicable, in tandem with molecular dynamics, to biochemical problems. We apply this MD/SOS-DFPT methodology [4,5,10,11] to an ensemble of N-methylacetamide complexes representing the three peptide linkages Gly-2, D-Val-8 and D-Leu-14, constructed from the configurations generated by a molecular dynamics trajectory of the gramicidin A (GA) channel embedded in a fully hydrated dimyristoylphosphatidylcholine (DMPC) bilayer. Such a combined MD/SOS-DFPT approach is advantageous. A molecular dynamics trajectory, generated with a computationally inexpensive empirical potential function, is used to sample the microscopic short-time-scale fluctuations. A limited number of instantaneous configurations are extracted to perform accurate SOS-DFPT calculations of the shift tensor. The channel formed by the simple pentadecapeptide GA, chosen for the present investigation, represents an ideal prototypical model for examining the variations in ^{15}N chemical shifts of a membrane protein. It has been the object of extensive experimental as well as theoretical studies [12,13]. In particular, its conformation in the membrane has been determined, based on solid state NMR from the observed ^{15}N backbone chemical shift and the N–H dipolar coupling [2].

2. Methodology

2.1. Microscopic systems

A molecular dynamics trajectory of an 8:1 DMPC:GA system with 44 wt% water was used to perform the conformational sampling. The molecular dynamics calculations have been described in detail elsewhere [4,5]. Briefly, the simulation system consists of 16 DMPC lipid molecules surrounding a central GA channel and solvated by 650 water molecules. The infinite membrane system was modeled by using hexagonal boundary conditions within the bilayer plane, along with periodic boundary conditions in the direction normal to the bilayer. The all-hydrogen potential function PARAM 22 of the CHARMM program was used for the gramicidin channel and for the DMPC [14,15]; the TIP3P potential was used for the water molecules [16].

To perform the DF calculations, it is necessary to select a reduced system with a reasonable number of atoms. This was accomplished by substituting three peptide bond linkages of the gramicidin backbone Gly-2, D-Val-8 and D-Leu-14, by N-methylacetamide molecules (NMA). Although larger fragments are probably needed for quantitative accuracy, the NMA molecule should provide a realistic first model for the electronic structure of the nitrogen in the amide plane and we think it is appropriate for the present study which focuses on the question of fluctuations. Similar atomic substitution methods have been used (see Ref. [17] and therein), in combined quantum mechanical and molecular mechanical simulations (QM/MM), in the sum of interactions between fragments computed ab initio procedure (SIBFA) and to study non-additive polarization effects on ion–peptide interactions. For example, the

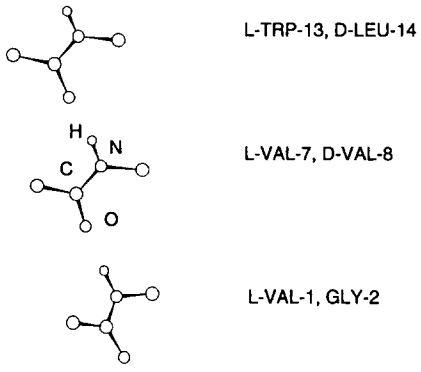

Fig. 1. Atomic substitution of three peptide linkages of the gramicidin channel backbone by three NMA molecules. All other atoms of the system, including the water molecules, were removed. The central NMA corresponds to the atoms of Val-7 (C_α, C, and O) and D-Val-8 (N, H, and C_α). The two other NMA molecules provide the hydrogen bonding neighbors characteristic of the gramicidin β-helical structure. The NMA at the bottom is constructed from the atoms of Val-1 (C_α, C, and O) and Gly-2 (N, H, and C_α); the NMA at the top is constructed from the atoms of Trp-13 (C_α, C, and O) and D-Leu-14 (N, H, and C_α).

central NMA was substituted for the atoms of Val-7 (C_α, C, and O) and D-Val-8 (N, H, and C_α), i.e. C_7^α was substituted by CH_3, C_7 by C, O_7 by O, N_8 by N, H_8 by H and C_8^α by CH_3.

To reproduce the two hydrogen bonding neighbors of the central NMA in the gramicidin β-helix structure, a second NMA was substituted for the atoms of Val-1 (C_α, C, and O) and Gly-2 (N, H, and C_α) and a third for the atoms of Trp-13 (C_α, C, and O) and D-Leu-14 (N, H, and C_α). All other atoms of the system, including the water molecules, were removed. Hydrogens of the two methyl groups were constructed with bond lengths of 1.107 Å and bond angles of 108.53 deg. There are 12 atoms in one NMA for a total of 36 atoms when hydrogen bonding was considered. The construction of the NMA systems is illustrated in Fig. 1. Ten different conformations were extracted from the 500 ps molecular dynamics trajectory. A first set of five conformations separated by 0.05 ps and a second set of five conformations separated by 50 ps were chosen to examine the chemical shift variations on two different time scales. The atomic coordinates of those ten conformations correspond to instantaneous snapshots as they occurred along the molecular dynamics trajectory (no conformational averaging was performed). We view them as simply revealing the nature of some of the possible fluctuations. Work towards gathering more conformations so that an average maybe calculated is in progress [18].

2.2. Chemical shift calculations

Because the solid state NMR time scale is much longer than that of rapid molecular motions, the chemical shift observed experimentally results from a time-average of the projected instantaneous second-rank shielding tensor [1] i.e.,

$$\sigma_{\text{obs}} = \left\langle \sum_{i=1}^{3} \left[\hat{\boldsymbol{n}} \cdot \hat{\boldsymbol{e}}_i(t)\right] \sigma_{ii}(t) \left[\hat{\boldsymbol{e}}_i(t) \cdot \hat{\boldsymbol{n}}\right] \right\rangle, \qquad (1)$$

where $\sigma_{ii}(t)$ and $\hat{\boldsymbol{e}}(t)$ are, respectively, the instantaneous magnitude and direction of the principal tensor components and $\hat{\boldsymbol{n}}$ is a unit vector in the direction of the magnetic field [1,2]. In solid state NMR experiments on membrane proteins, the chemical shift of site-specific ^{15}N labeled proteins is often measured with a magnetic field parallel to the membrane normal [1,2]. For D-Val-8, the magnitude and orientation of the three components of the ^{15}N chemical shift tensor were determined experimentally from powder spectra of the gramicidin channel [19]. The component magnitudes are obtained directly from the sharp discontinuities in the chemical shift powder pattern whereas its orientation relative to the C–N–H group is extracted by analyzing the dipolar-coupled ^{15}N chemical shift powder pattern using the heteronuclear dipolar interaction between ^{15}N and ^{13}C in doubly labeled samples [19]. The largest component of the tensor, σ_{33}, was found to have a magnitude of 201 ppm and an orientation approximately parallel to the N–H bond in the amide plane. Based on the ^{15}N–^{13}C dipolar interaction, an angle of 105° (typical of the polypeptide backbone) between the principal axis and the C–N bond was assumed [19]. The second component, σ_{22} (perpendicular to the amide plane) has a magnitude of 55 ppm, and the third σ_{11} (in the amide plane) a magnitude of 28 ppm [19].

[1] Here we use the notation σ (rather than δ) for chemical shift tensors following the experimental works [1,2].

If one sets the (admirable) goal of predicting experimental spectra from molecular dynamics, a rigid tensor approximation is very attractive. One simply 'ties' the experimental tensor determined from the powder sample to the molecular frame, runs the dynamics and calculates the appropriate average. However, the fixed orientation has to be defined relative to one or another feature of the (eventually moving) framework structure and there is no unique way of doing this. In a first attempt to see whether this is important we have considered two definitions of the backbone ^{15}N rigid chemical shift tensor. In the first definition (rigid tensor 1), the principal axis of the first component (201 ppm) is constructed in the H–N–C plane, with an angle of 105° relative to the N–C bond. The principal axis of the second component (55 ppm) is constructed perpendicular to the H–N–C plane and that of the third component (28 ppm) is obtained from a cross product of the first and second principal axes (i.e. $\hat{e}_3 = \hat{e}_1 \times \hat{e}_2$). In the second definition (rigid tensor 2), the direction of the first component is constructed in the H–N–C plane, with an angle of 19° relative to the N–H bond (i.e. using a standard peptide geometry). The direction of the second component is constructed perpendicular to the H–N–C plane and the third component is obtained from a cross product of the $\hat{e}_1$ and the $\hat{e}_2$ principal axes. Though the component magnitudes σ_{ii} were taken as the experimentally measured values [2] in both cases, the two rigid tensors differ when the configuration of the H–N–C peptide linkage deviates from the ideal geometry during the trajectory. The ^{15}N chemical shift in the direction normal to the membrane was calculated from an average over all the configurations of the 500 ps trajectory using the rigid tensor 1 and 2 described above. The calculated chemical shifts are 136 and 125 ppm on average with rms fluctuations on the order of 20 ppm for the rigid tensor 1 and 2, respectively. The experimental value is 145 ppm for D-Val-8 in a DMPC bilayer [20]. The difference in the average values of the two models, 11 ppm gives some measure of the uncertainties to be associated with this rigid tensor model when it is used to analyze a trajectory. Clearly, more information is needed on the structure dependence of the tensor components as the frame fluctuates.

The DF calculations of the ^{15}N chemical shift tensor were carried out with a new sum-over-states density functional perturbation theory (SOS-DEPT) [10,11] with the IGLO gauge choice (individual gauge for localized orbitals) [7] implemented in the deMon program [21–23]. The DF approach has vastly increased speed relative to post Hartree–Fock approaches, so that meaningful biomolecular models may be treated, yet attains high accuracy by incorporating exchange and correlation effects. We refer the reader to Malkin et al. [10,11] for results validating the SOS-DFPT method. The Perdew–Wang-91 (PW91) exchange–correlation potential [24–26] and the approximation Loc.1 SOS-DFPT [10,11] were used. For all of the calculations the basis sets IGLO-II of Kutzelnigg et al. [7] were employed. In order to obtain more precise molecular orbital coefficients and one-electron energies after reaching convergence during SCF iterations, one extra iteration was performed without fitting of the exchange–correlation potential and using an enlarged grid [10]. See Refs. [10,11] for further details.

To evaluate the chemical shift tensor components their absolute counterparts obtained from the SOS-DFPT calculations were subtracted from an isotropic reference value of 223.7 ppm corresponding to the isotropic chemical shift of a saturated $^{15}NH_4NO_3$ solution, which has been used as a reference in the experiments [27]. The value of −223.7 ppm was deduced from the chemical shift of $^{15}NH_4NO_3$ (−359.5 ppm) [28] relative to the absolute chemical shift $CH_3^{15}NO_2$ (−135.8 ppm) [29]. For example, the SOS-DFPT calculated principal values for the fifth configuration with three NMA were 207.1, 163.8, and 21.9 ppm, and those were adjusted to 16.6, 59.9 and 201.8 ppm, corresponding to σ_{11}, σ_{22}, and σ_{33}, respectively.

3. Results and discussion

Fig. 2 shows the magnitudes of the chemical shift components calculated with SOS-DFPT for all 10 configurations. The calculated component magnitudes are in reasonable agreement with the values determined experimentally from the powder sample [2]. The three components are correctly ordered. The largest fluctuations are on the order of 30%; most values lie much close. The largest variation of com-

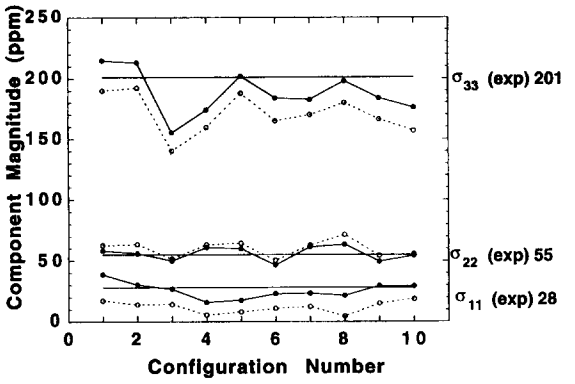

Fig. 2. Instantaneous value of the component magnitudes, $\sigma_{ii}(t)$, for the ^{15}N chemical shift tensor of D-Val-8 calculated with SOS-DFPT. The filled symbols are the results of the calculations with 3 NMA molecules, incorporating the effects of the β-helical hydrogen bonding. The open symbols are the results of the calculations with only the central NMA. The straight lines show the three experimental values determined from powder spectra [20].

ponent magnitude occurs for σ_{33} where fluctuations up to 70 ppm are observed. For the other two components, the deviations from the mean are relatively small. The observed chemical shift results from a time-average of the instantaneous values of the chemical shift in the direction normal to the membrane, $\sigma_{\|}(t)$. The results of calculations for the parallel chemical shift with rigid tensor 1 (reference N–C bond), rigid tensor 2 (reference N–H bond) and the SOS-DFPT calculations with both 1 and 3 NMA molecules are shown in Fig. 3. The contribution from each component of the tensor to the total parallel chemical shift can be explored. Fig. 4 shows that the dominant contribution to the instantaneous $\sigma_{\|}(t)$ arises from the component $\sigma_{33}(t)$. This is primarily due to the fact that all backbone N–H bonds of the β-helix (corresponding approximately to the direction of the first component of the tensor) are directed nearly parallel to the bilayer normal in the membrane-bound channel conformation.

No significant differences are observed between the short (0.05 ps) and long (50 ps) time scales. A comparison of the first five and the second five conformations shows that the chemical shift in the parallel direction is fluctuating by about the same amount and in the same directions between the first five and the second five conformations. This indicates that the rapid fluctuations in the instantaneous tensor are related to the fast motions taking place on the 50 fs time scale (first five conformations). The slower motions, taking place on the 50 ps time scale (last five configurations), do not lead to qualitatively different variations in the instantaneous chemical shift tensor. This is important because it suggests that the very fast small amplitude motions are sufficient to estimate the significant variations in the instantaneous value of the chemical shift tensor.

It has been suggested that hydrogen bonding can also influence the chemical shift of polypeptides and proteins [9]. The importance of hydrogen bonding can be considered by comparing the results of the SOS-DFPT calculations with the reduced systems with 1 and 3 NMA. The hydrogen bonding neighbors of D-Val-8 characteristic of the gramicidin β-helical structure are incorporated in the reduced systems with 3 NMA. It can be observed on Fig. 3, that the effect of hydrogen bonding results in a constant offset factor on the order of 13 ppm for σ_{33} and σ_{11} for the 10 configurations, σ_{22} being nearly unaffected. In a similar spirit, it can be suggested that a similar systematic shift would be observed for a larger number of configurations. Hence the hydrogen bonds are not overriding importance to the model; their inclusion can be considered to be fine tunning.

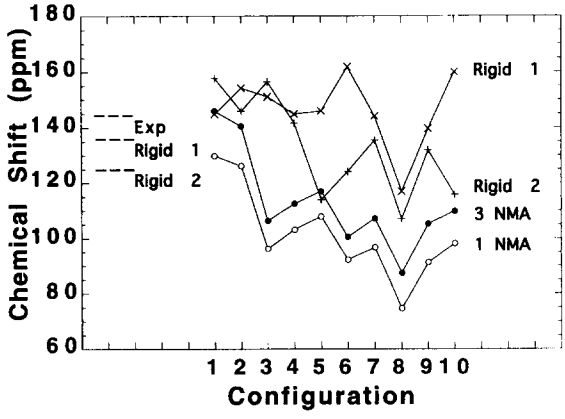

Fig. 3. The instantaneous value of $\sigma_{\|}(t)$, the ^{15}N chemical shift in the direction parallel to the membrane normal. The C–N and the N–H definitions of the rigid tensor, as well as the results of the SOS-DFPT calculations with 1 and 3 NMA molecules are shown. On the left side of the figure are shown the averages over the 500 psec trajectory using rigid tensor 1 and rigid tensor 2, respectively.

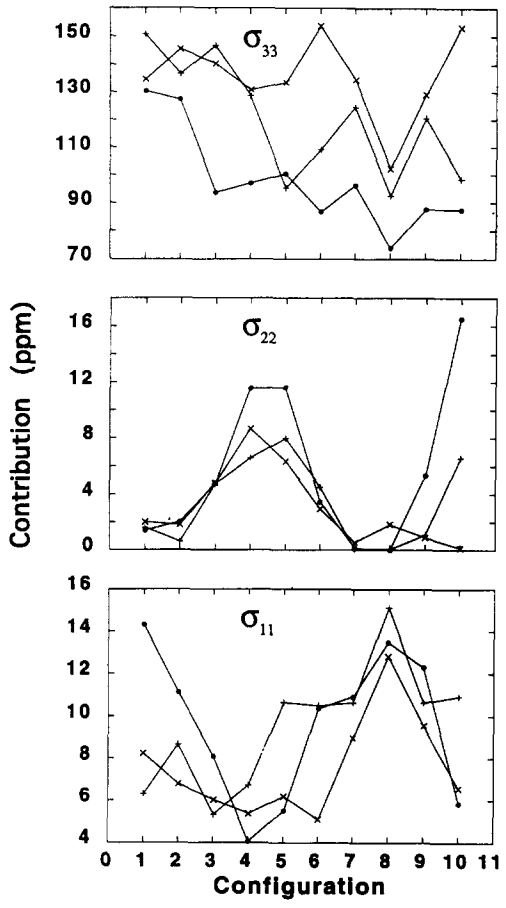

Fig. 4. Contribution of each component to $\sigma_{\parallel}(t)$, the instantaneous value of the ^{15}N chemical shift in the parallel direction, $\sigma_{ii}(t)(\hat{e}_i(t)\cdot\hat{n}_i)^2$. The dominant contribution is made by the first component.

The chemical shift within the GA:DMPC model, calculated on the basis of the rigid tensor approximation, may disagree with the DFPT chemical shift for two different reasons (apart from the approximations inherent in the reduced models and the computational method per se). Problems can arise from both inaccuracies in the component directions $\hat{e}_i(t)$ or variations in the magnitudes of the tensor $\sigma_{ii}(t)$. The chemical shift of configuration 6 provides an example of the first kind of problem in using rigid tensors. It is observed in Fig. 3 that the chemical shift parallel to the membrane normal differs from the SOS-DFPT value by almost 60 ppm. The error is not due to a change in the component magnitude since the component magnitude σ_{33}, shown on Fig. 2, does not differ markedly from the SOS-DFPT chemical shift for this configuration. The chemical shift calculated with the rigid tensor 1 is inaccurate because the component direction $\hat{e}_1$, constructed from the C–N bond, is in error. The bond angle involving the C–N–H is slightly distorted in configuration 6, as shown on Fig. 5, which leads to an error in the construction of the component direction $\hat{e}_1$. The chemical shift tensor of D-Val-8, determined experimentally from powder sample, assigned the σ_{33} component in the plane of the C–N–O atoms to an angle of 105° with respect to the C–N bond. In conformation 6, the component direction $\hat{e}_1$ makes an angle of 87° relative to the C–N bond. With the

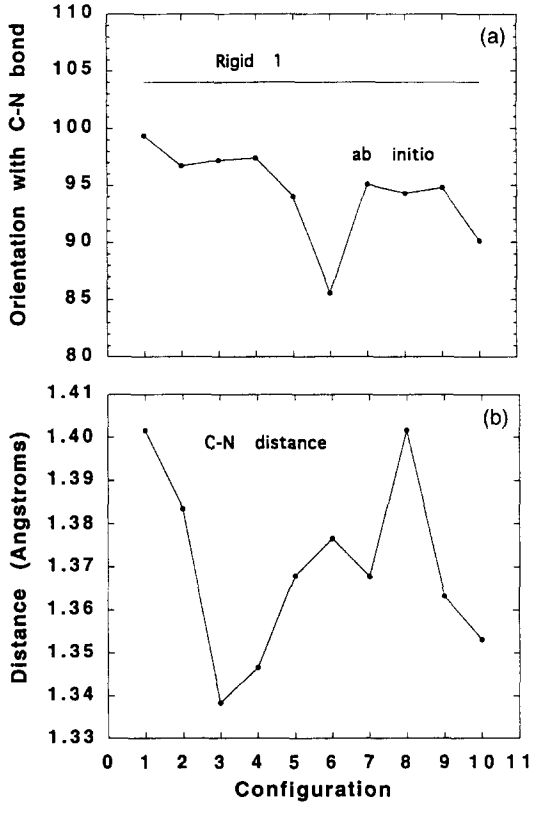

Fig. 5. Rigid tensor 1 is in error due to changes in the $\hat{e}_1(t)$ tensor orientation. (A) shows the angle between the SOS-DFPT 3 NMA $\sigma_{33}(t)$ component and the C–N bond. Also shown is the definition of 104° for this angle in the first rigid tensor. The second rigid tensor has an error due to changes in the magnitude of the $\sigma_{33}(t)$ component. (B) shows changes in the C–N bond length with configuration number.

rigid tensor 2, the chemical shift of configuration 6 is in better agreement with the SOS-DFPT calculation because the component direction, constructed in the C–N–H plane with an angle of 19° from the H–N bond, is more accurate. As discussed elsewhere [18], the reason is simply that the direction of the N–H bond is somewhat better correlated with the direction of the principal axis of the tensor component σ_{33}. The second source of error, that due to variations in the component magnitudes, $\sigma_{ii}(t)$, can be seen for the rigid tensor 2 in configuration number 3 of Fig. 3. That the source of error is due to magnitude and not orientation can be observed from Fig. 2. The variation of σ_{33} is related to fluctuations in the C–N bond length. In the case of configuration 3 the bond length is the shortest of all 10 configurations as can be seen in Fig. 5.

Although the present analysis suggests that rigid tensor 2 is somewhat better correlated with the DF calculations than rigid tensor 1, the resulting average chemical shift calculated from the 500 ps trajectory does not agree as well with the experimental data. The values are 136 and 125 ppm on average for the rigid tensor 1 and 2, respectively, while the experimental value is 145 ppm [20]. Different factors could have contributed to the disagreement with experimental data. For example, the chemical shift is known to be extremely sensitive to small variations of internal geometry, particularly bond lengths [9], and it is possible that rigid tensor 2 does not provide an accurate approximation of the tensor for particular distortions of the peptide unit. To illustrate the response of the component magnitudes and directions to the internal geometry of the peptide linkage, we calculated the first derivative of the chemical shift tensor of an isolated NMA using finite difference methods. The first derivative of σ_{33} with respect to the length of the C–N bond length is $+300$ ppm/Å (to lowest order the orientation of the components did not change). Small fluctuations can lead to significant variations in the chemical shift. During the 500 ps trajectory, the rms fluctuations are on the order of 0.03 Å for bond lengths and 3° for bond angles. However, despite the important sensitivity of the component magnitude to the internal geometry, it is possible that the effects of short-time-scale variations cancel on average. To clarify the influence of the rapid variations in the component magnitudes, it is assumed that the fluctuations of $\hat{e}_i(t)$ are uncorrelated with those of $\sigma_{ii}(t)$ in Eq. (1),

$$\sigma_{\mathrm{obs}} \approx \sum_{i=1}^{3} \langle [\hat{n} \cdot \hat{e}_i(t)]^2 \rangle \langle \sigma_{ii}(t) \rangle. \quad (2)$$

Expanding the tensor around the equilibrium geometry of the local molecular frame in terms of the generalized internal coordinates ζ_i, it may be expected that the time average of $\sigma_{ii}(t)$ is close to $(\sigma_{ii})_{(\mathrm{eq})}$,

$$\langle \sigma_{ii}(t) \rangle \approx (\sigma_{ii})_{(\mathrm{eq})} + \sum_i \left(\frac{\partial \sigma_{ii}}{\partial \zeta_i} \right)_{(\mathrm{eq})} \langle \Delta \zeta_i(t) \rangle, \quad (3)$$

because the deviation in the internal coordinates relative to the equilibrium geometry, $\langle \Delta \zeta_i(t) \rangle$, is zero on average. Non-linear contributions may also be important in the relation between the chemical shift and the internal geometry of the peptide bond. In fact, the C–N bond length of configuration 3 changed by 0.06 Å, while the corresponding change in σ_{33} was 50 ppm, larger than expected from the calculated first derivative of 300 ppm/Å.

The experiment determines the orientation of the tensor relative to the molecular frame by making use of the heteronuclear dipolar interaction between ^{13}C and ^{15}N in doubly labeled samples [19]. Due to the NMR time scale, the measured orientation represents an effective average over very fast small amplitude atomic fluctuations involving the C–N axis (see Eqs. (2) and (3) above). It is conceivable that the C–N based tensor 1, once averaged over rapid fluctuations, is more consistent with the tensor orientation determined experimentally from the heteronuclear dipolar interaction between ^{13}C and ^{15}N. An analogous experimental procedure based on the heteronuclear dipolar interaction between ^{15}N and ^{1}H can also be used to determine the tensor orientation [30,31]. Such experiments provide the corresponding tensor orientation average over the small amplitude fast atomic fluctuations involving the N–H axis. Since the SOS-DFPT calculations indicate that the instantaneous orientation of the principal axis corresponding to the dominant component (σ_{33}) is slightly better correlated with the N–H axis, it would be of interest to experimentally determine the orientation of the tensor with respect to the N–H axis for comparison. Other correlations may also be sought

and this is the subject of an extension, in progress, of the present work to sufficient conformations to allow a meaningful statistical average.

4. Conclusion

A combined MD/SOS-DFPT method approach has been used to examine the importance of rapid microscopic fluctuations for both the component magnitudes and the directions of the ^{15}N chemical shift tensor have been investigated by performing sum-over-states density functional perturbation theory (SOS-DFPT) for an ensemble of NMA complexes constructed from the configurations generated by a molecular dynamics (MD) trajectory of the gramicidin channel in a fully hydrated phospholipid bilayer. The component of the chemical shift tensor parallel to the membrane normal was calculated. We tested a rigid tensor approximation, in which the magnitude and orientation of the tensor components determined experimentally from a powder sample are attached to the molecular framework. Two different choices for the structural element to which the tensor is tied lead to significantly different average values for the shift. Further calculations with the SOS-DFPT quantum-chemical method indicate that fluctuations in both the tensor orientation and magnitude occur for some configurations (the experimental spectrum corresponds to an average over these short-time-scale fluctuations).

On the short time scale, problems with the rigid chemical shift tensor may arise from either variations in the component directions $\hat{e}_i(t)$ or in the magnitudes of the tensor $\sigma_{ii}(t)$. Accuracy in the orientation of the rigid tensor components depends on the choice of the local molecular frame. For example, the calculations show that the instantaneous orientation of the dominant tensor component σ_{33} is somewhat better correlated with the N–H vector than with the C–N vector. In particular, the large deviations observed in the case of a few configurations are avoided [18]. Nevertheless, the average deviation remains generally significant, which suggests that these simple constructions in terms of the C–N or N–H bonds are insufficient to account for the instantaneous value of the tensor. For some configurations, the component magnitude is changed due to fluctuations in the internal geometry of the local molecular frame (e.g., bond stretching). This type of variation is more difficult to treat because a characterization of the sensitivity of the tensor component magnitude with respect to the internal geometry is required.

Although the SOS-DFPT calculations suggest that the instantaneous direction of the σ_{33} component is slightly better correlated with the N–H bond than with the C–N bond, the average chemical shift calculated from the molecular dynamics trajectory using that definition is in worse agreement with the experimental data. Although the SOS-DFPT method represents a breakthrough in terms of the size of system that can be treated with high accuracy, current applications are limited to around 50 atoms. Nonetheless, the effect of including the nearest hydrogen bonding neighbors was determined. It is only on the order of 13 ppm.

In future work using the MD/SOS-DFPT approach, a larger number of configurations extracted from the trajectory will be analyzed. It should then be possible to extract correlations between the shielding tensor and the internal geometry of the amide group by performing a large set of SOS-DFPT calculations on small fragments extracted from the molecular dynamics trajectory. With these new techniques one can now envisage performing calculations on enough conformations to obtain statistically meaningful averages. The door seems open for the first-principles predictions of the NMR spectrum for gramicidin and other complex biosystems under experimental conditions. Further investigations of the ^{15}N and ^{13}C chemical shift and the influence of Na^+ in the gramicidin binding site are underway.

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