Frozen-Solution Conformational Analysis by REDOR Spectroscopy

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Conformational analysis of organic and biomolecular compounds are routinely performed by solution- or solid-state NMR spectroscopy. Each method has its inherent limitations: solution conformations are typically time-averaged and limited in precision, and are often inadequate for describing distributions of rapidly equilibrating structures. Solid-state conformations of polycrystalline materials can be determined with considerably greater precision, but are subject to polymorphism and have no reliable correlation with solution structures. A third approach involves a hybrid of the two methods in which molecules are trapped in low-energy conformations by freezing them in a glassy matrix. This should allow for a quantitative analysis of discrete conformations and their relative populations within a static ensemble. Frozen-solution conformational analysis (FrSCA) was first validated by Long and Tycko for a helix-forming peptide in frozen aqueous solutions using magic-angle spinning (MAS) exchange spectroscopy.[1] Here we show that FrSCA can be applied to organic compounds by rotational-echo double resonance (REDOR) spectroscopy, a widely used method for measuring heteronuclear distances (up to 6 Å for $^{13}C-^{15}N$ spin pairs) with resolutions on the order of 0.1 Å or less.[2,3] Applications of REDOR include the conformational analysis of ligand molecules bound to receptor proteins[4] and the global conformational analysis of proteins and other biopolymers in frozen solution,[5] but its use in statistical conformational analysis has yet to be demonstrated.

Experiments were performed on a 400-MHz CMX spectrometer with a 9.4-T wide-bore magnet and a 5-mm triple-resonance MAS probe. Data were acquired using a standard REDOR pulse sequence,[6] enhanced by $^1H-^{13}C$ cross-polarization (CP) transfer and time-proportional phase-modulated (TPPM) decoupling (strength ∼84 kHz).[7] Samples of 2-^{13}C,^{15}N-glycine (**1**)[8] and ^{13}C-methyl β-D-^{15}N-acetylglucosamine (**2**)[9] were prepared as 0.7 and 0.4 M solutions in 95% D_2O to ensure good CP transfer without introducing additional T_2 broadening. These solutions were rapidly frozen in the sample rotor at −80 °C while spinning at 700 Hz; REDOR data was then acquired at rotor speeds of 4.5−5.0 kHz over a period of 30 and 240 rotor cycles for **1** and **2**, respectively. A final REDOR curve was constructed from the division of the ^{15}N-refocused data set (S_R) by the unperturbed data (S_0). Solutions of **1** and **2** showed negligible changes in ^{13}C and ^1H chemical shifts or T_1 relaxation times as a function of concentration, and could thus be considered as independent two-spin systems.

$^+H_3N-^{13}CH_2CO_2^-$

1   **2**
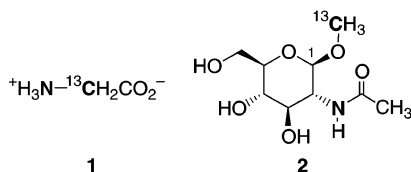
Studies were first conducted on frozen aqueous solutions of conformationally invariant **1** to determine the experimental uncer-

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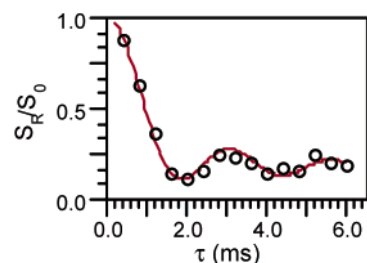
Figure 1. REDOR data for 2-^{13}C,^{15}N-labeled glycine (**1**) in frozen 95% D_2O at a MAS frequency of 5 kHz (open circles), and best fit of data based on a single $^{13}C-^{15}N$ coupling (red).
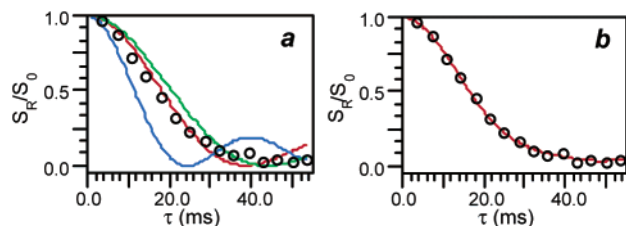

Figure 2. REDOR data for ^{13}C-methyl β-D-^{15}N-acetylglucosamine (**2**) in frozen 95% D_2O at a MAS frequency of 4.5 kHz (open circles), with least-squares fits based on one or two rigid dipolar couplings. (a) REDOR curves based on a single d_{C-N} value of 3.55 Å (blue), 4.13 Å (red), and 4.31 Å (green). (b) REDOR curve based on two d_{C-N} values (3.55 and 4.31 Å).

tainty in frozen-solution REDOR analysis (see Figure 1). Nonlinear least-squares analysis using the analytical formulations developed by Mueller[10] based on a single dipolar coupling yielded an optimized C2−N2 distance of 1.510 Å with a 95% confidence limit of 0.014 Å. This value is in excellent agreement with those measured from solid-state glycine powders using related NMR methods (1.505−1.52 Å)[11,12] but are longer than that measured by single-crystal X-ray diffraction of α-glycine (1.474 Å).[13,14]

FrSCA of methyl β-aminoglucoside **2** was performed to determine the conformational profile of its glycosidic (C1−O1) bond. Glycosidic linkages have a defining role in the secondary structures of carbohydrates, but analysis of their solution conformations has proven to be nontrivial.[15] The C1−O1 bond is considered to prefer a geometry close to the *gt* conformer in which the glycosidic substituent is approximately gauche to the O5 ring oxygen.[16] The "*exo*-anomeric" conformational preference has been widely assumed in carbohydrate secondary structures,[17] but to the best of our knowledge it has not been experimentally quantified for simple *O*-glycosides. This presented an opportunity to measure conformational distributions using FrSCA.

Frozen-solution REDOR data on **2** was acquired as described above, then fitted against several different models. Least-squares analysis based on a single C−N dipolar coupling gave a poor fit, whereas analyses involving two couplings provided a much closer fit (see Figure 2).[18] The two-state model yielded C_{Me}−N2 (d_{C-N})
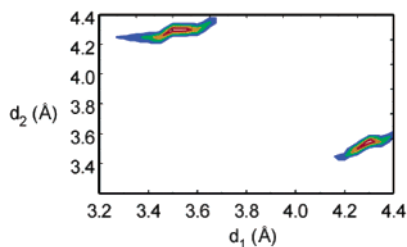
Figure 3. Contour plot of least-squares fits for two-conformer distributions, in which d_1 is varied by increments of 0.05 Å. Each contour level represents a 20% change in variance (σ_v^2).
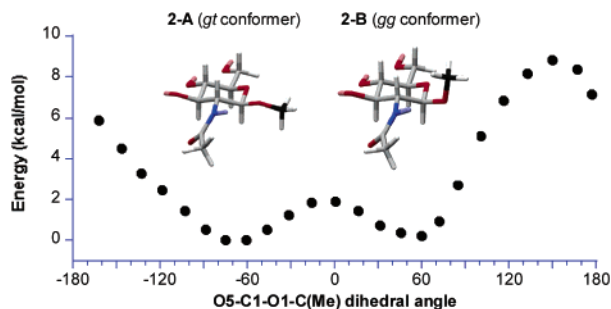
Figure 4. AM1 calculations of the relative conformational energies of **2** as a function of glycosidic dihedral angle (ϕ).

distances of 4.31 ± 0.06 Å and 3.55 ± 0.08 Å, with fractional populations of 0.68 and 0.32, respectively. To validate the accuracy of the two-conformer model, additional fits were performed in which one C–N distance (d_1) was fixed at values between 2.8 and 4.4 Å, the range of possible distances. Two minima were found which correspond to the results of the least-squares fit using two couplings (see Figure 3).

The frozen-solution conformational analysis of **2** was also corroborated with an independent study based on computational methods (see Figure 4).[19] Semiempirical AM1 calculations were used to minimize energies of gas-phase conformations with torsionally fixed dihedral angles about the glycosidic bond, yielding two relative minima close to the *gt* and *gg* conformers in a 60:40 ratio (**2-A**: $\phi = -75°$; **2-B**: $\phi = +60°$), in good agreement with the experimental observations.[18–20] Internuclear C_{Me}–N2 distances after geometry optimization were found to be 4.10 and 3.45 Å, respectively, both similar to the values obtained by REDOR. The computed C–N distances are based on the average bond lengths derived from X-ray crystallography and thus compare quite favorably with the slightly longer distances obtained from the REDOR measurements.

In conclusion, FrSCA offers an attractive alternative to solution-based NMR methods of conformational analysis, with its superior resolution and straightforward method of sample preparation. Other solid-state NMR techniques are also likely to be applicable to the frozen solution state, such as rotational resonance (^{13}C–^{13}C distances)[21] and angle-dependent correlations of chemical-shift

tensors.[22] FrSCA may be especially useful for studying the native conformations of molecules and materials in highly amorphous environments, such as gels or biological tissues and matrices.

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