Communication

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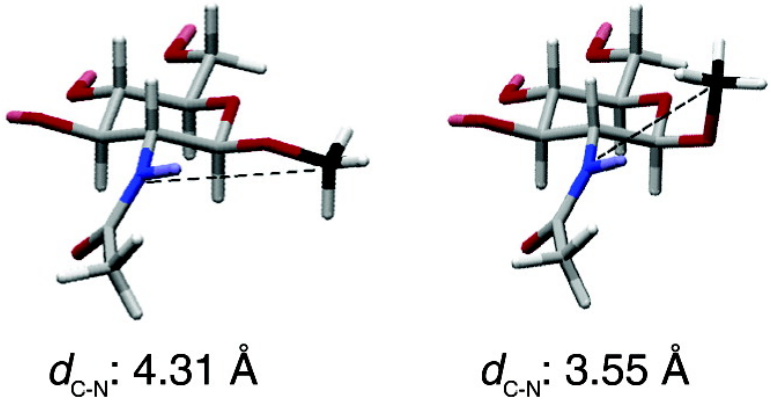

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Frozen-Solution Conformational Analysis by REDOR Spectroscopy

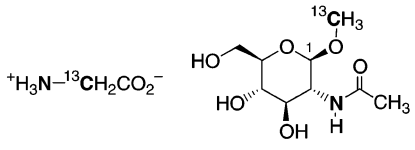
Luis J. Smith,† Fabien P. Boulineau, Daniel Raftery,* and Alexander Wei*

Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907-2084

Received June 5, 2003; E-mail: alexwei@purdue.edu

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 $^{1}H-^{13}C$ 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 ^{1}H chemical shifts or T_1 relaxation times as a function of concentration, and could thus be considered as independent two-spin systems.



1 **2**

Studies were first conducted on frozen aqueous solutions of conformationally invariant **1** to determine the experimental uncertainty 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]

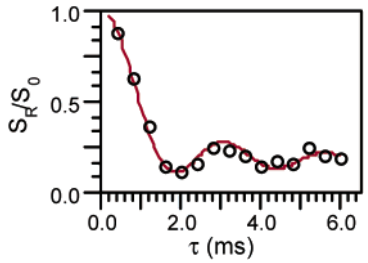


Figure 1. REDOR data for 2-^{13}C,^{15}N-labeled glycine (**1**) in frozen 95% D_2O at a MAS frequency of 5 kHz (open circles), and best fit of data based on a single $^{13}C-^{15}N$ coupling (red).

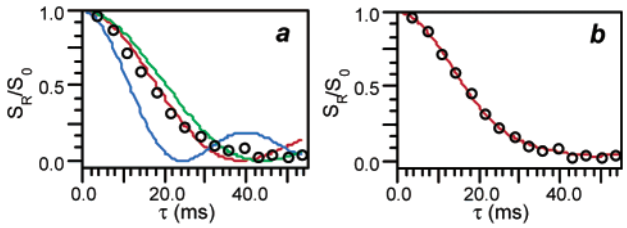


Figure 2. REDOR data for ^{13}C-methyl β-D-^{15}N-acetylglucosamine (**2**) in frozen 95% D_2O at a MAS frequency of 4.5 kHz (open circles), with least-squares fits based on one or two rigid dipolar couplings. (a) REDOR curves based on a single d_{C-N} value of 3.55 Å (blue), 4.13 Å (red), and 4.31 Å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

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})

† Present Address: Clark University, Department of Chemistry, 950 Main St., Worcester, MA, 01610.

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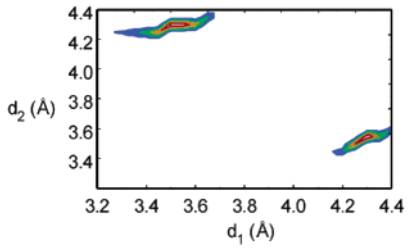

Figure 3. Contour plot of least-squares fits for two-conformer distributions, in which d_1 is varied by increments of 0.05 Å. Each contour level represents a 20% change in variance (σ_v^2).

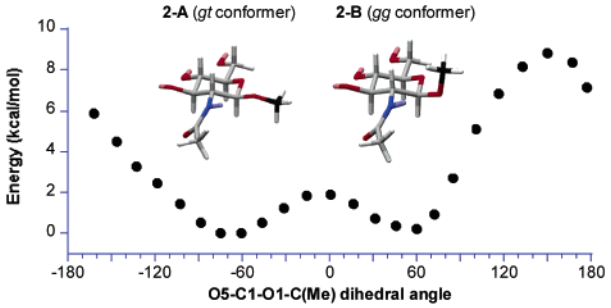

Figure 4. AM1 calculations of the relative conformational energies of **2** as a function of glycosidic dihedral angle (ϕ).

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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Supporting Information Available: Conditions for REDOR data analysis, and parameters for AM1 geometry optimizations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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