

## Determination of a Hydroxyl Conformation in Aqueous Xylose Using Neutron Scattering and Molecular Dynamics

Philip E. Mason,<sup>†</sup> George W. Neilson,<sup>‡</sup> John E. Enderby,<sup>‡</sup> Marie-Louise Saboungi,<sup>§</sup> and John W. Brady<sup>\*,†</sup>

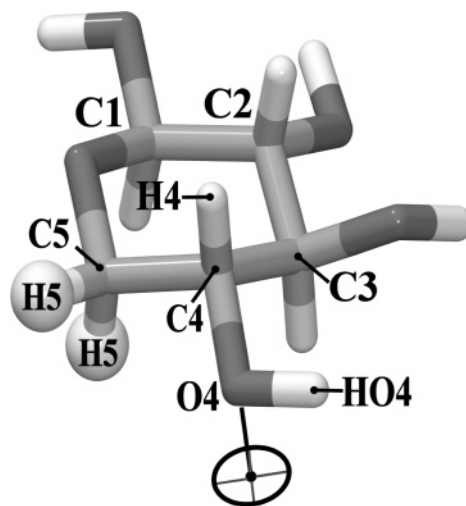
Department of Food Science, Stocking Hall, Cornell University, Ithaca, New York 14853,  
H. H. Wills Physics Laboratory, University of Bristol, BS8 1TL, U.K., and  
Centre de Recherche sur la Matière Divisée, 1 bis rue de la Férollerie, 45071 Orléans, France

Received: October 4, 2005; In Final Form: November 26, 2005

Recently, it was shown using structural neutron diffraction with isotopic substitutions (NDIS) measurements, combined with molecular dynamics simulations, that in an aqueous solution of D-xylose the hydroxyl group on the C4 position does not significantly occupy the position trans to the H4 atom. Here, a similar combination of NDIS and MD studies is described which uses D-xylose deuterated at the C5 position to further characterize this hydroxyl conformation as being trans to the C5 atom, as predicted by constrained MD simulations, confirming the previous study.

Sugars are vital in many biological, chemical, and engineering processes, and the conformations of sugars in solution are of growing interest, as carbohydrate derivatives are increasingly used in designing drugs and vaccines. Unfortunately, there are relatively few methods available for studying the structure of sugars in aqueous solution. A previous paper<sup>1</sup> has described the combination of molecular dynamics (MD) simulations and neutron diffraction with isotopic substitution (NDIS)<sup>2,3</sup> experiments to characterize the conformation of the hydroxyl group on the C4 carbon of D-xylopyranose in aqueous solution. This hydroxyl group is expected to have three intrinsic minima: trans to the C5, H4, or C3 atoms (hereafter called the *t*-H4, *t*-C3, or *t*-C5 positions, respectively; see Figure 1). The NDIS measurement was performed on solutions of D-xylose with a single H/D substitution label on the H4 position and was compared to MD simulations conducted under identical conditions of concentration and anomeric composition, with the C4 hydroxyl group constrained to be in the *t*-H4, *t*-C3, or *t*-C5 positions. This comparison suggested, in agreement with unconstrained MD simulations, that the *t*-H4 position was preferentially unoccupied, while there was insufficient resolution in the experimental data to distinguish between the very similar *t*-C3 and *t*-C5 positions, although the unconstrained MD simulation suggested that the *t*-C5 conformation was preferred.<sup>1</sup>

Similar MD studies of D-glucopyranose have shown that the conformation of the exo-cyclic hydroxymethyl group and the hydroxyl groups of glucose are almost completely insensitive to concentration over the range 1–5 molal (molar ratios of between 55 and 10 water molecules per sugar).<sup>4</sup> It was also found that the number of hydrogen bonds that glucose formed was insensitive to concentration over the same concentration range; however, as the concentration of the sugar increases, the



**Figure 1.** The atom naming used in the current study. The H5 atoms are the ones substituted with deuterium in the NDIS study. These data are used to examine the position of the OH group on the C4 atom. This OH group has three expected minima, trans to the H4, C5, and C3 groups, respectively.

number of hydrogen bonds to other glucose molecules increases at the expense of hydrogen bonds to water, as would be expected. These studies additionally suggest that, given the poor contrast of NDIS experiments using singly substituted sugars at lower concentrations, higher glucose concentrations would yield better results.

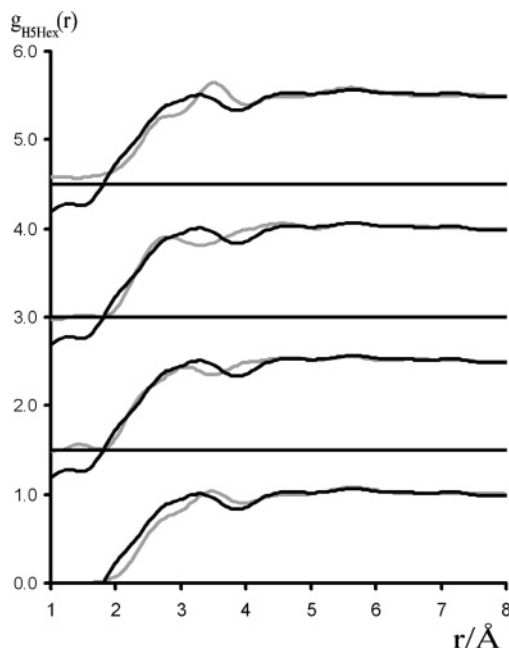
The present report describes a similar study of D-xylose labeled with deuterium at the H5 positions. The general method used here, which compares constrained MD simulations to NDIS experimental data, is similar to that used previously. However, in the present case, the experiment was performed at a 5 molal concentration rather than the 3 molal of the previous xylose study. In addition, in the present study, both of the H5 atoms

\* jwb7@cornell.edu.

<sup>†</sup> Cornell University.

<sup>‡</sup> University of Bristol.

<sup>§</sup> Centre de Recherche sur la Matière Divisée.



**Figure 2.** The black line is the experimental determined function  $g_{\text{HsubHex}}(r)$ , while the gray line is the same function calculated from MD. From bottom to top, the gray lines represent the free simulation and that constrained in the *t*-H4, *t*-C3, and *t*-C5 positions, respectively.

are substituted simultaneously, while in the previous experiment, only the single H4 atom was labeled. These two factors mean that the contrast in the present experiment is significantly higher than in the previous study (the contrast of  $g_{\text{H5Hex}}(r)$  in the current study is 39.9 mb versus 15.6 mb for  $g_{\text{H4Hex}}(r)$  in the previous study). Comparison of MD simulations at 3 and 5 molal showed a negligible change in the rotational populations of the C4 hydroxyl group.

Simulations were performed in TIP3P<sup>5</sup> solution using CHARMM<sup>6</sup> at 3 and 5 molal concentrations, with the C4 hydroxyl group successively constrained in each of the *t*-H4, *t*-C3, and *t*-C5 positions using a harmonic restraining potential with a force constant of 200 kcal mol<sup>-1</sup>. In addition, an unconstrained simulation was performed to determine the simulated conformational equilibrium. The MD methods used were identical to those described previously.<sup>1</sup> In each case, the function  $g_{\text{H5Hex}}(r)$ , the radial distribution function for exchangeable hydrogen atoms Hex around the labeled H5 positions, was calculated directly from the simulations.

The NDIS method used for the determination of  $g_{\text{H5Hex}}(r)$  has been described previously.<sup>2,3</sup> Figure 2 shows the experimentally determined function  $g_{\text{H5Hex}}(r)$  and the same function determined from MD. In each case, the molecular dynamics function has had the identical resolution applied to it as in the experimental measurement.<sup>4</sup> It was found that the function  $g_{\text{H5Hex}}(r)$  was devoid of sharp features with the exception of the intramolecular H5–HO4 correlation. Much of the sharpness of this correlation is lost because of the limitations of the resolution of the instrument; however, the method is still sensitive to the position of this peak. In the case of the *t*-H4 and *t*-C3 conformations, two peaks are observed, since although the hydroxyl group of the C4 is constrained into one position, the distances to each of the H5 atoms are different. These double peaks are at 2.5 and 3.1 Å for both the *t*-H4 and *t*-C3 conformations, while for the *t*-C5 conformation, only one peak is observed at 3.5 Å. After the resolution limit of the experimental measurement has been imposed, this results in one peak for each position at 2.8, 2.8, and 3.5 Å, respectively, for

the *t*-H4, *t*-C5, and *t*-C3. There are two main factors contributing to the function  $g_{\text{H5Hex}}(r)$ . The first is the general hydration of the H5 atoms, while the second, sharp feature is due to the intramolecular H5–HO4 correlation. The general hydration has the appearance of a largely featureless phased step function between 1.9 and 4 Å, while the sharper correlation is a single discrete feature. Because of the difference in the sharpness of these features, each has little effect upon the other, e.g., the step function does not significantly affect the position or the appearance of the molecular correlation. The experimental measurement is individually sensitive to both of these features. In the comparison of MD and experimental  $g_{\text{H5Hex}}(r)$  functions, these features may be examined separately. It was found that the unconstrained simulation, which had a conformer population of approximately 0.75 *t*-C5, 0.25 *t*-C3 for both the  $\alpha$  and  $\beta$  anomers, gave the best fit to the experimental data, while the simulations with the C4 hydroxyl group constrained in the all-*t*-C5 and all-*t*-H4 conformers gave significantly poorer fits for the position of the H5–HO4 peak. A further simulation with the C4 hydroxyl group constrained to be eclipsed to the H4 atom (*e*-H4, between the *t*-C3 and *t*-C5 positions) gave an even better fit to the experimental data. From the MD data, this is not necessarily surprising, since in the unconstrained simulation, the C4 hydroxyl group occupied mostly the *t*-C5 and *t*-C3 conformations but had a significant population at the *e*-H4 position. The experimental data implies that this property, predicted by the MD, is in reality stronger than the MD would suggest. However, while the unconstrained simulation does a reasonable job at predicting the correct position for the H5–HO4 peak position, it seems to show a phase difference of between 2 and 4 Å. This phase discrepancy is on a longer length scale than the phase differences due to the poor peak position prediction of the constrained simulations, which typically show a more pronounced phase discrepancy over only a 1 Å range (suggesting that a sharp feature is being predicted poorly). The longer length scale of this phase discrepancy in the unconstrained simulation is probably due to the poorer prediction of the general hydrophobic hydration of the aliphatic CH<sub>2</sub> group at the C5 position using the TIP3P water model.

The unconstrained MD simulations using the CHARMM potential function gave a reasonable agreement with the experimental population, but since constrained simulations were also used to interpret the diffraction data, the method would not be dependent on how well the molecular mechanics force field reproduced this profile, and any energy function could have been used with equal success. The present study, in agreement with the previous study of the H4 substitution of xylose,<sup>1</sup> suggest that the C4 hydroxyl group occupies mostly the *t*-C5 and *t*-C3 positions, although the distribution of these states probably allows a significant population in the *e*-H4 positions and mostly favors the *t*-C5 position. It is possible that this conformation is preferentially occupied because it offers the possibility of an internal hydrogen bond between the OH and the O at the C3 position.

**Acknowledgment.** The authors gratefully acknowledge the assistance of G. Cuello of the Institut Laue-Langevin. This project was supported by grant GM63018 from the National Institutes of Health and by a grant of beam time from the ILL.

## References and Notes

- (1) Mason, P. E.; Neilson, G. W.; Enderby, J. E.; Sabounji, M.-L.; Brady, J. W. *J. Am. Chem. Soc.* **2005**, *127*, 10991.
- (2) Enderby, J. E. *Chem. Soc. Rev.* **1995**, *24*, 159.

- (3) Neilson, G. W.; Enderby, J. E. *J. Phys. Chem.* **1996**, *100*, 1317.
- (4) Mason, P. E.; Neilson, G. W.; Enderby, J. E.; Saboungi, M.-L.; Cuello, G.; Brady, J. W., unpublished results.

- (5) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926.
- (6) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.