Quantitative analyses of NMR J-coupling ensembles in oligosaccharides: Glycosidic linkage conformational populations using circular statistics

Wenhui Zhang1, Toby Turney1, Qingfeng Pan2, Matthew Hadad3, Atticus Coscia3, Reagan Meredith4, Xiaocong Wang3, Robert J. Woods3, Mi-Kyung Yoon1, Ian Carmichael1 and Anthony S. Sierrani1

1Department of Chemistry and Biochemistry, and 2Radiation Laboratory, University of Notre Dame, Notre Dame, IN 46556-5670 USA; 3Omicon Biochemicals, Inc., South Bend, IN 46617-2701 USA; 4Complex Carbohydrate Research Center, University of Georgia, Athens GA 30602 USA

ABSTRACT

Groups (ensembles) of J-couplings in saccharides often report on the same conformational element. This redundancy confers key advantages to J-based conformational analysis but also introduces the potential for quantitative relationships between their magnitudes and signs, and molecular structure, are available. To develop an approach, an array of eleven disaccharides (methyl glycosides) containing β-(1→4)-linkages was prepared that display systematic structural variation in the vicinity of the internal glycosidic linkage. The disaccharides were selectively labeled with 13C and one of two strategies, to facilitate measurements of J-couplings across their linkages. At least ten J-couplings (\(J_{\varphi\psi}\), \(J_{\varphi\eta}\), \(J_{\psi\eta}\), \(J_{\eta\varphi}\), \(J_{\eta\psi}\), \(J_{\psi\varphi}\)) in the vicinity of the linkage are sensitive to its conformational torsion angles, \(\varphi\) (C1'-O1-C1-C2'), \(\psi\) (C1-O1-C2-C3'), and \(\eta\) (C1-C2-O1-C3'). J-coupling ensembles were measured experimentally, and DFT parameterization was combined with Frenkelian theory and circular statistics encoded in the newly developed MA'T program to calculate experiment-based rotamer populations of the glycosidic linkage in each disaccharide. Uniform models of \(\varphi\) and \(\psi\) were obtained for each disaccharide to display the same conformational behavior for \(\varphi\) but different behaviors for \(\psi\), as determined exclusively from J-based experimental constraints. These NMR experiments, RFT calculations and statistical analyses were undertaken to test and validate the method, and allow for comparisons to be made to MD simulations of the same systems. Results show that modification of the MD method may be required in some cases to accurately reproduce the experimental results (e.g., inclusion of oxygen lone-pair orbitals in saccharide simulations).

A. Redundant NMR J-Couplings

B. 13C-Labeled Disaccharide Array

C. NMR Spectroscopy

D. X-Ray Crystallography

E. Molecular Dynamics

F. Density Functional Theory Calculations

G. MA'T Treatment of NMR J-Couplings

H. Statistical Modeling

J-coupling ensembles and DFT parameterization were combined with Frenkelian theory and circular statistics to determine the conformational populations of O-glycosidic linkages. A J-coupling, \(J_{\psi\varphi}\), sensitive to a specific torsion angle \(\varphi\) can be modeled as eq. [1], where \(p(\varphi)\) is the probability at \(\varphi\) and \(J_{\psi\varphi}(\varphi)\) is the coupling constant associated with \(\varphi\). This modeling of assumptions about the mean position or the degree of dispersion about this position, this is the case for eq. [2]. While eq. [2] allows for the production of multi-state models with relatively few parameters, it is limited by the inherent assumptions made about the collection of allowable conformational populations in solution. This methodology is for the first assumption is zero bias about the preferred values of \(\varphi\) which takes the form of one to three perfectly staggered rotamers. This treatment is especially unsuitable when a particular J-coupling ensemble is lower than the expected value as well as when the model is unable to accurately reproduce the experimental results.

Supported by NSF CHE 1402744 and NIH SBIR R01GM083028C

Equation parameterization was performed for the linkages in Group II, 11 (Group II) and 12 (Group III), giving 18 equations. When equations for the specific disaccharides were very similar, they were combined to give one general equation. Prior work has shown that trans-glycoside J-couplings depend primarily on either \(\varphi\) or \(\psi\) but, in the cases of the A-series, heuristics were applied to model torsion angles. Modeling the dual dependencies of \(J_{\psi\varphi}\) values requires equations that can, within the parameter space, accurately describe the significant features in the data. Some of these features, however, may not be practically important because they occur in regions of \(\varphi\)-\(\psi\) space that are predicted by the potential energy surfaces to be poorly sampled in solution. To circumvent this problem, only J-couplings in structures falling under a relative energy barrier of 10 kcal/mol were used in equation parameterization.