

Short communication

Solid-state ^{13}C NMR investigations of the glycosidic linkage in α - α' trehalose

P. Zhang, A.N. Klymachyov, S. Brown, J.G. Ellington, P.J. Grandinetti *

Department of Chemistry, The Ohio State University, 120 W. 18th Avenue, Columbus, OH 43210-1173, USA

Received 15 May 1998; revised 13 July 1998; accepted 13 July 1998

Abstract

The sugar trehalose is known to play a central role in the desiccation tolerance of many organisms. Essential to trehalose's role are its glass forming abilities and ability to directly interact with lipid molecules. Detailed information on the structure and dynamics of glassy trehalose and its interactions with lipids, however, have been elusive. We have used solid-state NMR and ab initio quantum mechanical methods (Gaussian 94) in order to characterize the possible molecular conformations of trehalose. Using a simplified structure (2-(tetrahydropyran-2-yloxy) tetrahydropyran) as a model we have calculated the energy and ^{13}C magnetic shielding parameters as a function of the two glycosidic torsion angles. Combining ab initio derived maps and using the ^{13}C lineshape as constraints we were able to construct the torsion angle distribution map for α - α' trehalose. We believe measurements of ^{13}C isotropic chemical shift and other solid-state NMR tensor parameter distributions in combination with ab initio methods can prove useful in identifying sources of structural disorder in glassy trehalose. By monitoring these structural distributions new information about the membrane surface associative properties of trehalose and other sugars should be accessible. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: α - α' trehalose; Glass structure; ^{13}C solid-state NMR; Ab initio methods

Recently, there has been interest in extending the shelf life of certain drugs and biomaterials by using α - α' trehalose as a stabilizing material [1,2]. A glassy form of this disaccharide protects biomaterials such as liposomes and proteins during freeze drying. A major difficulty in improving these methods is that the molecular mechanism by which α - α' trehalose interacts and stabilizes dry membranes and proteins is not well understood [1,3,4]. While the glassy state of the sugar is central to its stabilizing ability, it also

presents a major obstacle to researchers since the traditional scattering techniques cannot provide detailed structural information on amorphous solids. On the other hand, spectroscopic techniques like solid-state NMR have proven to be successful probes of local structure in glasses [5,6]. We report here an approach for determining molecular conformations of amorphous trehalose using ^{13}C cross polarization magic angle spinning (CP/MAS) NMR combined with ab initio quantum chemistry methods.

In Fig. 1 is the ^1H -decoupled ^{13}C CP/MAS NMR spectrum of crystalline α - α' trehalose dihydrate (Sigma). It exhibits six partially resolved peaks with a well-separated (1 ppm FWHM) peak for the $\text{C}_1(\text{C}'_1)$ carbon at 93 ppm (shifted 20 ppm downfield from

* Corresponding author. Tel.: +1-614-292-6818; Fax: +1-614-292-1685; E-mail: grandinetti.1@osu.edu

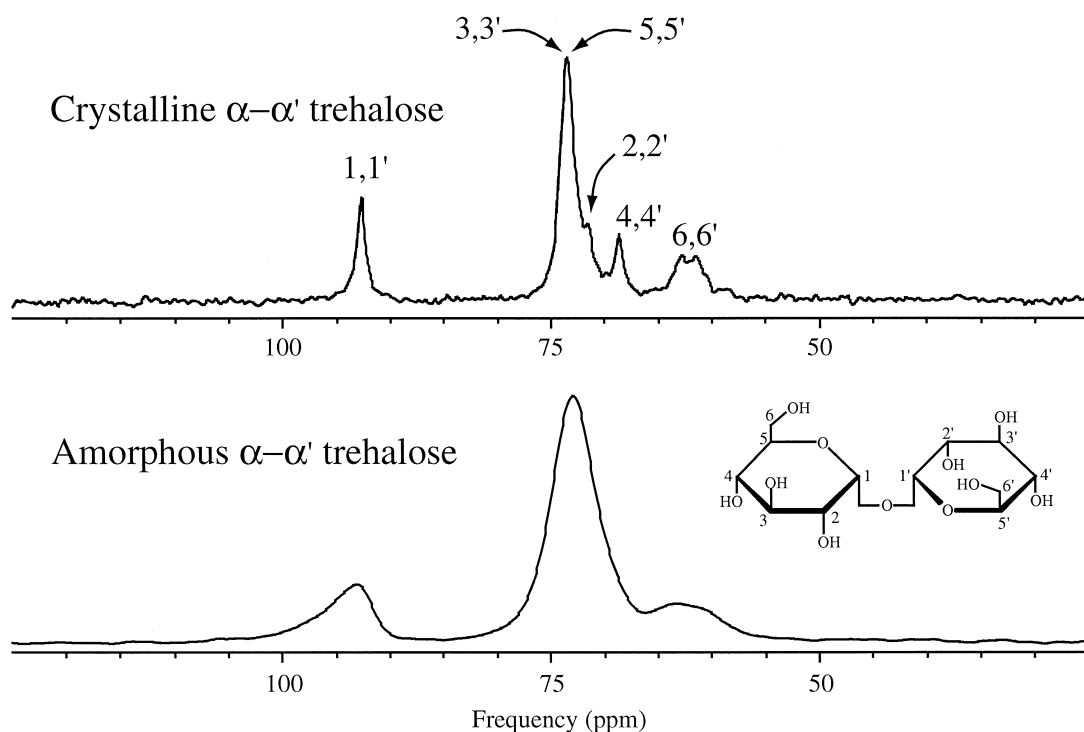


Fig. 1. ^1H -decoupled ^{13}C CP/MAS spectra of crystalline and amorphous α - α' trehalose.

the remaining peaks). On going from the crystalline to amorphous compound (obtained by evaporation from an aqueous solution and verified by X-ray diffraction) we observed a significant (over 5 ppm) asymmetrical broadening of the C_1 resonance, similar to that reported by Gidley and Bociek for amorphous α -(1–4) glucans [7]. Because α - α' trehalose consists of two rigid pyranose rings, the main source of broadening arises from the variation in the torsion angles of the glycosidic linkage. Based on optical rotation data for α - α' trehalose in solution Duda and Stevens [8] suggested that the glycosidic torsion angles are in the region of 62° and the extent of their conformational excursions is extremely limited. Because of fast molecular motions in solution, however, only averaged conformations are observed. Thus, reconstruction of the distribution in torsion angles is problematic, at best. By contrast, the glassy state is structurally rigid, yet retains many of the structural distributions present in the liquid state. Thus, it is the ideal state in which to investigate the

conformational degrees of freedom accessible to the trehalose molecule.

While there have been several attempts to relate the ^{13}C chemical shift and glycosidic conformation for groups of related carbohydrates [7,9–12], all those relations were empirically derived and had limited success in relating chemical shift to structure [13]. With the aim of obtaining a more robust mapping between ^{13}C chemical shift and glycosidic torsion angle, we performed a series of ab initio calculations [14] on the α - α' trehalose structural skeleton, 2-(tetrahydropyran-2-yloxy) tetrahydropyranin. Using 6-31(d) basis sets the energy and ^{13}C chemical shift conformation maps were constructed and are shown in Fig. 2. The ^{13}C shielding computations were performed using the GIAO method [15] as implemented in Gaussian 94 [14]. The calculated energy map (Fig. 2a) is in fair agreement with the one constructed by Tvarovska and Vaclavik [16] with the semiempirical PCILO (perturbative configuration interaction using localized orbitals) method.

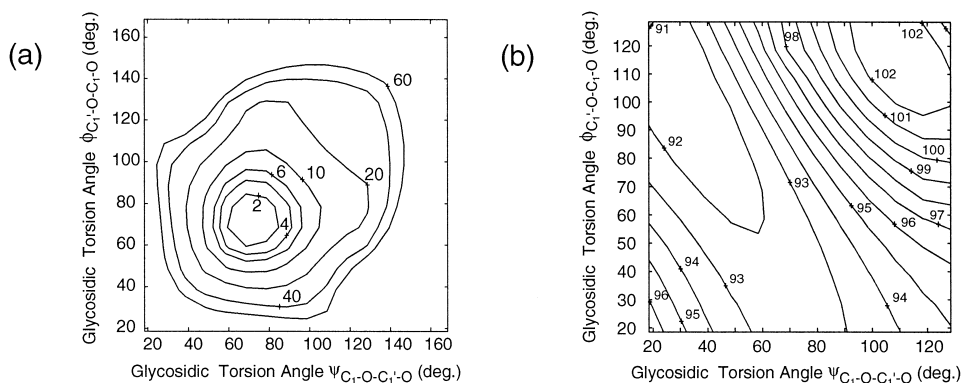


Fig. 2. (a) Energy surface (kJ/mol) and (b) ^{13}C isotropic chemical shift surface (ppm) for 2-(tetrahydropyran-2-yloxy) tetrahydropyran as a function of glycosidic torsion angles. Calculations were performed with Gaussian 94 [14] using 6-31(d) basis set. The glycosidic bridging oxygen angle was fixed at the trehalose dihydrate crystalline value of 115.9° . For the NMR calculations C_1 chemical shift of crystalline trehalose (93 ppm) was used as a reference.

Given $p(\phi, \psi)$, the two-dimensional distribution of glycosidic torsion angles, and $\delta(\phi, \psi)$, our chemical shift map, the ^{13}C spectrum of the C_1 resonance can be calculated according to

$$I(\delta) = \sum_i \exp\left\{-\left(\frac{\delta - \delta_i(\phi, \psi)}{\Delta\delta}\right)^2\right\} p(\phi, \psi) \Delta\phi\Delta\psi, \quad (1)$$

where $\Delta\delta$ is the homogeneous linewidth of the C_1 resonance. Certainly there will be many possible torsion angle distributions that can map into one C_1 lineshape. Therefore, we have assumed that there will be a Boltzmann distribution of conformations governed by the ab initio derived energy map in Fig. 2. The best fit of this model to the experimental lineshape is shown Fig. 3. The model reproduces the skewed C_1 lineshape remarkably well. The distribution of torsion angles consistent with this best fit lineshape is given in Fig. 4. The distribution is centered about $(\phi, \psi) = (76^\circ, 76^\circ)$ with standard deviation of $(17^\circ, 17^\circ)$ compared to the crystalline values of 60.8° and 60.1° for anhydrous α - α' trehalose [19] and 61.8° and 74.8° for the dihydrate [17,18] (all angles are given in C–O–C–O notation). The one-dimensional projections of the distribution are asymmetric and have skewness of 0.5. These results predict a greater extent of conformational excursions in glassy trehalose than predicted from optical rotation data for trehalose in solution [8].

A complication in our model is that the calculated conformational energy map does not take cooperative intermolecular interactions into account, which are expected to be important in glassy materials.

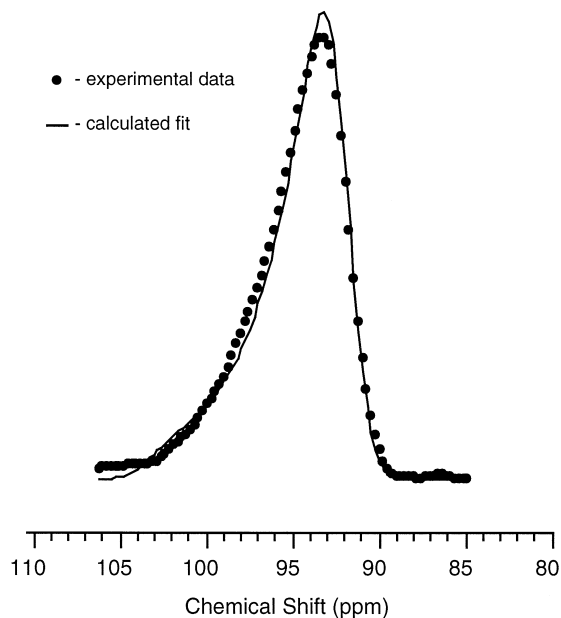


Fig. 3. Experimental ^{13}C CP/MAS spectrum of glassy trehalose (C_1 peak is shown) and least-square fit with $T = 716.8$ K. $\Delta\delta$ was fixed at 1.5 ppm (i.e., approximated as the linewidth in the crystalline compound).

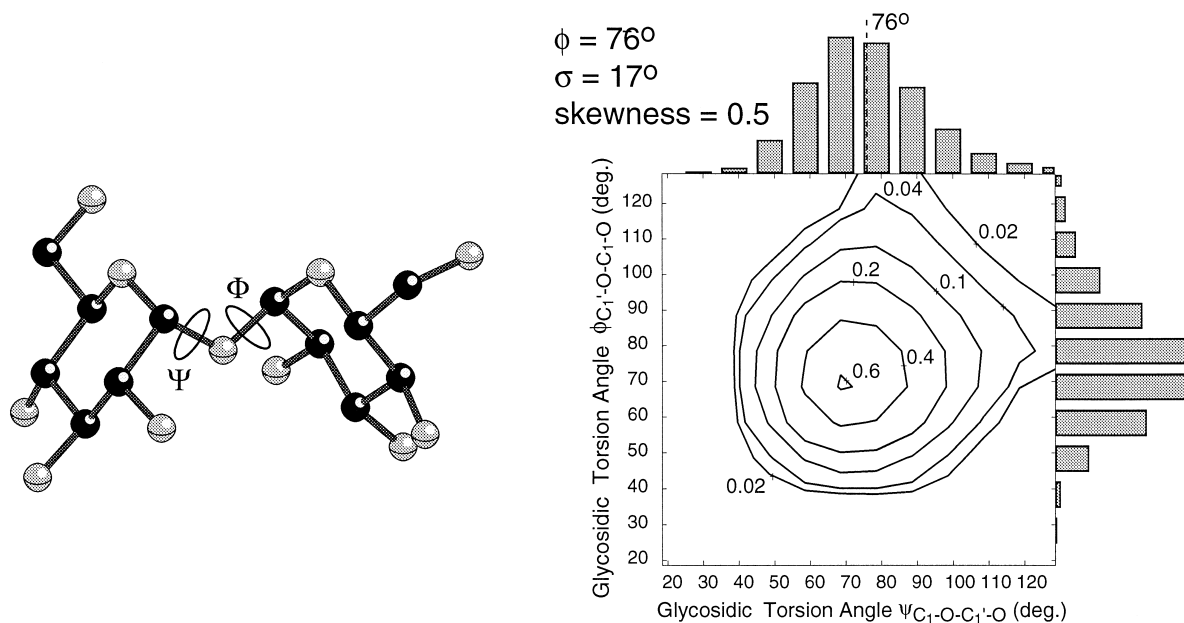


Fig. 4. Distribution of glycosidic torsion angles in glassy trehalose as obtained from the energy surface (Fig. 2a) assuming a Boltzmann distribution with the best fit temperature of 716.8 K. The probability density units are derived from the normalization conditions and reported in 10^{-3} deg^{-2} .

Indeed, the temperature required to obtain the fit in Fig. 3 is 716.8 K. This is considerably higher than the glass transition temperature values of 310 or 350 K for α - α' trehalose dihydrate or anhydrous α - α' trehalose, respectively. Alternatively, we can think of our fit as having the temperature fixed at 310 K with the conformational energy map scaled down by a factor of ~ 0.43 . Our interpretation of this model is that the cooperative intermolecular interactions are isotropic and lower the conformational energy barriers of the α - α' trehalose molecule, thus making more conformations accessible than would have been predicted on the basis of the gas phase conformational energies.

To check our assumption that the distribution of the torsion angles is indeed the main source of spectral broadening we repeated the ab initio calculations varying only the C–O–C bridging glycosidic angle. Energy and ^{13}C chemical shift maps (one dimensional in this case) were constructed. By simulating ^{13}C spectrum we found that not more than 0.5 ppm of spectral broadening can be attributed to the dispersion in bridging glycosidic angle. This supports the hypothesis that the variation in glycosidic

torsion angles are mainly responsible for the chemical shift dispersion of C_1 carbons in glassy trehalose.

In general, we expect conformational changes which may occur when α - α' trehalose binds to a membrane surface to have an observable effect on the C_1 ^{13}C resonance. Thus the C_1 ^{13}C CP/MAS isotropic lineshape should serve as a useful probe of qualitative conformational changes in the glycosidic torsion angles as trehalose interacts with membrane surface. While we have shown that quantitative torsion angle distribution information can be obtained using ab initio methods and the assumption of a Boltzmann distribution of conformers, some caution may be warranted when extending this approach to trehalose–lipid systems since the conformational energy map of trehalose interacting with a lipid surface may deviate from the gas phase conformational energy map. A more promising but experimentally challenging approach to extract the glycosidic torsion angles would be to doubly ^{13}C labeled the glycosidic linkage and measure the full ^{13}C chemical shift and dipolar spin interaction tensors and their relative orientations. Further work along these lines is in progress.

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