Carbohydrate Research 345 (2010) 1469–1481

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Experimental and theoretical electron density distribution of α,α-trehalose dihydrate

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ARTICLE INFO

Article history:
Received 16 January 2010
Received in revised form 10 March 2010
Accepted 14 March 2010
Available online 17 March 2010

Keywords:
Carbohydrate
Crystal structure
Disaccharide
Energy
Gradient vector field
Mycose

ABSTRACT

α,α-Trehalose is of interest because of its cryoprotective and antidessicant properties, and because it possesses various technical anomalies such as ^{13}C NMR spectra that give misleading indications of intramolecular structural symmetry. It is a non-reducing disaccharide, with the glycosidic oxygen atom shared by the anomeric carbon atoms of the two glucose rings, and is therefore subject to a proposed 'overlapping' *exo*-anomeric effect. We report here a study of the electron density of trehalose with X-ray diffraction and quantum mechanics calculations, similar to a recent study of sucrose, also a non-reducing molecule. In particular we studied the electron density around the glycosidic linkage and the hydrogen bonding with both deformation density and Atoms in Molecules (AIM) analyses. A total of 129,952 single crystal X-ray intensity measurements were collected on α,α-trehalose dihydrate to a resolution of $\sin\theta/\lambda = 1.18$ Å^{-1} at 100 K and refined with an aspherical multipole model to a final agreement factor of $R_1 = 0.0160$. Wavefunctions were calculated at three levels of theory. Redistribution of electron density due to anomeric effects was reduced in trehalose, compared to sucrose. Five new C–H···O hydrogen bonds were confirmed with bond critical points and bond paths from AIM analyses, as were the previously proposed O–H···O hydrogen bonds.

1. Introduction

According to the 1912 Handbook of Sugar Analysis,[1] trehalose (α-D-glucopyranosyl-(1,1)-α-D-glucopyranose) was discovered by Wiggers in fungi that grow on rye and other grasses. Also called mycose or mushroom sugar, it constitutes 10–25% of the dry weight of mushrooms. Its more common name arises from its prevalence in trehala manna, a secretion from beetles in Syria and Iran. Not found in most higher plants, trehalose is present in substantial quantity in the desert resurrection plant, *Selaginella lepidophylla.* It is now well known as a protectant against frost and dehydration and is commonly used in the laboratory for the preservation of biomolecules,[2,3] but proposed mechanisms of protection are controversial.[4] Trehalose also can prevent oxidation of fatty acids in food and human skin.[5] Three isomers of 1,1-linked glucopyranosyl residues exist: α,α-trehalose, α,β-trehalose, and β,β-trehalose, with α,α-trehalose being so much more prevalent that it is often simply called trehalose.

Trehalose is also of continuing interest because of unexpected technical aspects. For example, the CP/MAS (cross polarization, magic angle spinning) NMR spectra of the dihydrate and anhydrous forms contradict the crystallographic findings in that the two glycosidic linkage torsion angles ϕ (O5–C1–O1–C1′) and ϕ' (O5′–C1′–O1–C1) of the anhydrous form are nearly identical, but it still gives two well-resolved peaks for C1 and C1′.[6] On the other hand, trehalose dihydrate, which has distinctly different linkage torsion angles, gives a CP/MAS spectrum with only a single C1 peak. Molecular modeling studies have been controversial as well, with some empirical force field studies suggesting that the preferred conformation of the isolated (gas-phase) trehalose molecule is not consistent with the *exo*-anomeric effect.[7,8] The idea was that aqueous solution disrupted a strong intramolecular hydrogen bond that stabilized the proposed gas-phase form, allowing other factors to stabilize the solution phase form from which the various crystals grew. Subsequent quantum mechanical (QM) studies showed that this non-*exo*-anomeric form, with ϕ and $\phi' = 180°$ was not the most stable form in the gas phase, but forms other than those seen in crystals were favored at some levels of theory.[9] Other modeling studies predicted the crystal structure conformations.[10–12]

In 1972, results were published from four determinations of the crystal structure of the dihydrate, all in good agreement.[13,14] In 2008, the structure was determined again with modern methods, at room temperature, as part of a study of hydrates.[15] In addition, around 40 linkage geometries exist in crystal structures of related molecules. All these linkages conform to

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doi:10.1016/j.carres.2010.03.017

the *exo*-anomeric effect, that is, the values of ϕ and ϕ' are in the range of 45.1–91.4°. None has intramolecular, inter-residue O–H···O hydrogen bonds that were predicted by some modeling methods.

Besides providing information based on atom positions, high-resolution single-crystal X-ray diffraction measurements can be used to map the electron density distribution in crystalline solids.[16,17] The electron density distribution provides detailed three-dimensional information on the electronic structure of molecular solids, and careful comparisons between experimental and theoretical results indicate that experimental results are comparable in accuracy to large basis set ab initio theoretical calculations.[18,19] Electron densities are often interpreted in terms of the 'deformation density'. It is obtained by subtracting the electron density of a 'promolecule' that is composed of independent, overlapped, spherical atoms from the electron density determined by a multipole refinement of the X-ray data.[16,17]

Both experimental and theoretical electron densities can also be analyzed using Atoms in Molecules (AIM) concepts developed by Bader.[20] AIM analyses initially focus on determining the critical points in the electron density. They are found at nuclear centers, at the saddle point along the ridge of electron density connecting bonded atoms, at the centers of rings of bonded atoms, and in the centers of cages of bonded atoms. The magnitude of the electron density at the bond critical points (BCPs), $\rho(r_b)$, correlates with bond order. Also of interest is the Laplacian of the electron density, $\nabla^2\rho(r_b)$, at the BCP. Covalent bonds have large negative values of $\nabla^2\rho(r_b)$, while hydrogen bonds and other closed shell interactions typically have smaller positive $\nabla^2\rho(r_b)$ values.

Recently, a charge-density (electron-density) analysis of sucrose was published[21] based on low-temperature X-ray diffraction measurements and on QM calculations. The experimental electron density was interpreted with deformation density methods, and AIM theory was applied to both the X-ray experiments and the theoretical calculations. The crystal structure of sucrose has been determined even more often than that of trehalose, including one study by neutron diffraction. Despite the well-established structure, new information emerged from the charge density study of sucrose. One of the most intriguing results was the experimental energies of the different hydrogen bonds. Other results included the various electron density parameters, charges for the individual atoms, and the range of atomic volumes.

Other relevant previous experimental electron density determinations of β-DL-arabinose[22] and 1,4-dioxane and *trans*-2,5-dichloro-1,4-dioxane[23] focused on the deformation densities of C–O covalent bonds and oxygen lone pairs associated with the anomeric effect. Our similar efforts were undertaken to gain the information given above for trehalose. Trehalose, like sucrose, is a non-reducing disaccharide and its two monosaccharide residues have head-to-head bonding and could be expected to have overlapping[24] *exo*-anomeric effects (in Ref. 24, the long C–O–C–O–C–O–C sequences were excluded in their survey because it was assumed that these extended sequences might perturb the statistics of the more ordinary C–O–C–O–C sequences). Unlike the fairly labile sucrose molecule, the glycosidic linkage in trehalose is quite stable.[25,26] The delocalization, as proposed in QM interpretations of the general anomeric effect, of the lone pairs of electrons on the shared glycosidic oxygen atom should be observable in these charge density studies.

The present redetermination of trehalose is considerably more accurate and a re-examination of the hydrogen bonding is warranted, as well as reporting of the C–H···O hydrogen bonds that were not considered previously. Compared to the sucrose study, the AIM analysis of the QM results has been expanded to include intermolecular interactions.

2. Experimental measurements and theoretical calculations

2.1. Data collection and conventional refinement

Single crystals of α,α-trehalose dihydrate were obtained by recrystallization from an aqueous solution. A colorless crystal with dimensions $0.60 \times 0.50 \times 0.45\ \text{mm}^3$ was mounted on a Bruker SMART 1K CCD three-circle diffractometer. The sample was cooled to 100(2) K with a stream of cold N_2 gas. The X-ray incident beam was generated with a standard focus Mo target X-ray tube, graphite monochromator and 0.8 mm collimator, providing illumination that was uniform within ±5% throughout the sample volume.

X-ray intensities were measured with ω scans at 0.30° increments over a range of 180° at a crystal-to-detector distance of 3.50 cm. A total of 11 scans were collected, six scans at 60 s per frame with the detector at $2\theta = 35°$ and $\phi = 0°$, 20°, 90°, 110°, 180°, and 270°, and five scans at 120 s per frame with the detector at $2\theta = 80°$ and $\phi = 45°$, 135°, 165°, 225°, and 315°. Since the N_2 stream was coaxial with the ω scan axis, and the χ angle was fixed at 54.74°, the crystal mount remained at a fixed angle with respect to the cold stream throughout all the scans. Raw intensities were integrated with SAINT[27] and an empirical absorption correction was applied with SADABS.[27] The ratio of the minimum to the maximum transmission factors used in the absorption correction was 0.985. The structure was solved and refined with a spherical atom model with SHELXTL (Table 1).[28] Results were also analyzed with PLATON.[29]

2.2. Multipole refinement

To obtain the experimental electron density distribution for the dihydrate, the diffraction data were refined with the Hansen–Coppens multipole deformation density model[30] as implemented in XD2006.[31] In this model, the density of each atom is represented by spherical core and valence densities calculated from Hartree–Fock atomic wavefunctions and an aspherical density contribution consisting of an expansion in Slater-type radial functions and spherical harmonic angular functions (Eq. 1):

$$\rho_{\text{atom}}(\mathbf{r}) = \rho_{\text{core}}(\mathbf{r}) + P_{\text{valence}}\kappa^3\rho_{\text{valence}}(\kappa\mathbf{r}) + \Sigma_l \kappa'^3 R_l(\kappa'\mathbf{r})\Sigma_m P_{l,m} Y_{l,m}(\theta,\phi) \quad (1)$$

The spherical core density contribution, $\rho_{\text{core}}(\mathbf{r})$, is fixed, while the valence density contribution, $\rho_{\text{valence}}(\mathbf{r})$, may be modified by the refinement of a valence population parameter, P_{valence}, and an expansion/contraction parameter, κ. The aspherical density contributions are modified by refinement of a multipole population parameter, $P_{l,\text{m}}$, for each density deformation function in the expansion up to the hexadecapolar ($l = 4$) level, and by refinement of a second expansion/contraction parameter, κ'. Thus, in addition to the three positional parameters and six anisotropic thermal displacement parameters for each atom, the multipole model introduces up to an additional 27 parameters to describe the electron distribution of each atom.

To reduce the number of variables, some parameters are constrained during the refinement, for example, for chemically equivalent atoms[21] or to impose symmetry on the density based on the local chemical environment.[32] For trehalose, no chemical constraints were imposed on the model. However, initial non-crystallographic site constraints were imposed. For example, for the hydroxyl O atoms, the local coordinate system and refined deformation parameters were chosen to impose a mirror plane symmetry in the plane defined by the O atom and the C and H atoms bonded to it. For the other oxygen atoms (including the two water oxygen atoms), two mirror planes were imposed: one in the plane