DFT application in conformational determination of cellobiose

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ARTICLE INFO

Article history:

Received 14 August 2014

Received in revised form 8 December 2014

Accepted 16 December 2014

Available online 23 December 2014

Keywords:

Cellobiose

Chemical shift

DFT

Potential energy surface

Dihedral angle

ABSTRACT

The potential energy of β -cellobiose in water, as a function of the H1'C1'O'C4 (ϕ) and C1'O'C4H4 (ψ) dihedral angles, is investigated using DFT methods and three energy minima (*syn- ϕ /syn- ψ* , *anti- ϕ /syn- ψ* , and *syn- ϕ /anti- ψ*) are identified. It is found that the hydrogen bonding and the H...H repulsion are vital for the energy variation of cellobiose. The geometry structure varies significantly when cellobiose is surrounded by 52 water molecules. NMR chemical shift calculations suggest that the C4 (C1') chemical shifts (δ) are sensitive to the conformational changes, with the values of 80.4 ppm (103.3 ppm) at *syn- ϕ /syn- ψ* , 70.0 ppm (98.9 ppm) at *anti- ϕ /syn- ψ* , and 75.6 ppm (95.3 ppm) at *syn- ϕ /anti- ψ* . The chemical shifts detection of characteristic positions is helpful for the determination of conformations, especially for those with equivalent energies. The chemical shielding tensor analysis shows that for C1', the directions and amplitudes of two shielding components (σ_{22} and σ_{33}) vary distinctly with ϕ and ψ whereas for C4, σ_{33} of C4 remains unchanged, but σ_{22} varies by 13 (26) ppm from *syn- ϕ /syn- ψ* to *anti- ϕ /syn- ψ* (*syn- ϕ /anti- ψ*). The distinctive C4 and C1' chemical shifts at different conformations suggest that they can be useful in (ϕ , ψ) dihedral angle determinations.

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1. Introduction

The conformational flexibility of saccharides is pivotal for the molecular recognition by biological targets, such as lectins, enzymes, and antibodies.^{1–4} Structural determination is crucial for understanding these recognition processes. Conformational analyses of saccharides, especially lactose and cellobiose, have been carried out by using X-ray crystallography,⁵ NMR⁶ (such as NOE,⁷ J-coupling constant,^{8,9} residual dipolar couplings (RDCs),^{10–12} and paramagnetic pseudocontact shifts (PCSs)¹³), and theoretical calculations.^{14–24} The cellobiose or lactose flexibility mainly arises from two dihedral angles ϕ (H1'–C1'–O'–C4) and ψ (C1'–O'–C4–H4), which in principle vary from -180° to 180° (Fig. 1). Theoretical studies have identified three stable conformers for cellobiose or lactose, namely *syn- ϕ /syn- ψ* ($\phi \sim 30^\circ$, $\psi \sim 0^\circ$), *anti- ϕ /syn- ψ* ($\phi \sim 180^\circ$, $\psi \sim 0^\circ$), and *syn- ϕ /anti- ψ* ($\phi \sim 30^\circ$, $\psi \sim 180^\circ$). But it is not clear which conformer is the most stable one, depending on the computational method used.^{18,21–23} NMR studies have suggested that the *syn- ϕ /syn- ψ* conformer is the most stable one in solution.^{7,17,25–27} But the population of the other two states cannot be agreed on and is actively debated. An early work using RDCs combined with molecular dynamics (MD) simulations shows that *syn- ϕ /syn- ψ* is the sole conformation of lactose.¹⁰ But a more recent work using RDCs and

PCSs demonstrates that *syn- ϕ /syn- ψ* only accounts for 75% of total population and the remaining 25% is from *anti- ϕ /syn- ψ* and *syn- ϕ /anti- ψ* .¹³ Similarly for cellobiose, Wildmalm and co-workers concluded that the population of *syn- ϕ /syn- ψ* is about 93%,^{17,25} while Bell and his colleagues suggested that *syn- ϕ /syn- ψ* is the dominant conformation and the flexibility of cellobiose is very limited.¹² All NMR measurables such as RDCs or J-couplings reflect the population average of the quantities with respect to different conformations. Extraction of the population of different states relies on the measurement accuracy and the property of these quantities, specifically their sensitivity to the conformational differences. Apparently, identifying more NMR measurables that are sensitive to carbohydrate conformations will help to determine the population with better accuracy.

The relationship between chemical shifts and structures is well studied in proteins. Theoretical calculations have been successfully performed in both NMR shielding tensors^{28–30} and chemical shifts^{31–35} to explore the structural and environmental effects. The chemical shifts are extremely sensitive to structural changes.³⁶ The accurately predicted ¹³C chemical shifts^{37–39} provide a powerful measure for the protein structure validation. It is likely that a similar correlation between chemical shifts and carbohydrate structures exists as well though such a study has not been carried out systematically.

In this work, we explored the relationship between ¹H, ¹³C chemical shifts, and (ϕ , ψ) dihedral angles of β -cellobiose by using

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Figure 1. Molecular structure of α - and β -cellobiose with atomic numbering (ϕ : H1'C1'O'C4, ψ : C1'O'C4H4).

quantum mechanics DFT calculations.^{18,40} Our results show that the ^{13}C chemical shifts change considerably with the (ϕ , ψ) dihedral angles, while the variation of ^1H chemical shifts is smaller. The three stable conformers, *syn- ϕ /syn- ψ* , *anti- ϕ /syn- ψ* , and *syn- ϕ /anti- ψ* , have distinctive ^{13}C chemical shifts especially for C1' and C4 which thus can be used to distinguish the glycoside (ϕ , ψ) conformations.

2. Computational methodology

The calculations were performed with the Gaussian 09 program package.⁴¹ The solvation effect was included using the self-consistent reaction field (SCRF) method employing the polarizable continuum model (PCM)⁴² with a dielectric constant of 78.5. The NMR calculations were performed employing the gauge independent atomic orbital (GIAO)^{43,44} approach. Tetramethylsilane (TMS) was used as the reference molecule.

The multi-standard approach (MSTD) has a low dependence on the level of theory employed during the GIAO NMR calculation.⁴⁵ It is employed for the ^{13}C NMR chemical shift (δ_{calc}^x) calculation according to the following equation:

$$\delta_{\text{calc}}^x = \delta_{\text{ref}} - \delta_x + \delta_{\text{ref}} \quad (1)$$

where σ_{ref} and σ_x are the NMR isotropic magnetic shielding values for the reference compound and the corresponding nucleus respectively, computed at the same level of theory, and δ_{ref} is the experimental chemical shift of the reference compound in deuterated chloroform ($\delta_{\text{ref}}(\text{TMS}) = 0$; $\delta_{\text{ref}}(\text{Methanol}) = 50.41$).

To search for the optimum DFT and basis set combination, the ^1H and ^{13}C chemical shifts (δ) of α - and β -cellobiose in the *syn- ϕ /syn- ψ* conformation were calculated using B3LYP,⁴⁶ M062X, and WB97XD functionals at the 6-311+G(d) basis set level, as well as the PW1PW91⁴⁷ method at the 6-311+G(d), 6-311++G(d,p), 6-311++G(3df,3pd), Aug-cc-pVDZ, and Aug-cc-pVTZ basis set levels, after the energy minimization using the same method. The IR spectra are analyzed and the primary hydrogen bond stretching modes are assigned. The potential energy surface and the chemical shifts of cellobiose as the function of ϕ , ψ were determined with an angle interval of 10° at the PW1PW91/Aug-cc-pVDZ level.

The solvent is also explicitly modeled with the cellobiose surrounded by 52 water molecules. The water molecules are added with the O...O distance of 3.0 Å between the terminal hydroxyl (O1 and O4') and water molecule. Other water molecules are filled all around the cellobiose with the length of O...O between water molecules ~ 3.0 Å.

3. Results and discussion

3.1. Optimum DFT method for chemical shift calculations

To find out which DFT functional gives the best predicted chemical shifts for cellobiose at the *syn- ϕ /syn- ψ* conformation, B3LYP, M062X, PW1PW91, and WB97XD methods were tested with the

basis set of 6-311+G(d), and the results are listed in Table 1. The best functional is PW1PW91, with the root mean square deviation of carbon (δ_{CRMSD}) and hydrogen (δ_{HRMSD}) chemical shifts from the experimental values of 2.70 ppm and 0.19 ppm, respectively. The experimental and computational data were also fitted using the linear equation (Fig. S1). The PW1PW91 functional is the best with a correlation coefficient $R^2 = 0.994$ and a slope of 1.026 for ^{13}C chemical shifts and a correlation coefficient $R^2 = 0.898$ and a slope of 1.008 for ^1H chemical shifts.

To identify the optimum basis set, the chemical shift calculations were then performed with the PW1PW91 functional at the 6-311++G(d,p), 6-311++G(3df,3pd), Aug-cc-pVDZ, and Aug-cc-pVTZ levels, respectively. The δ values are collected in Table 2. It can be seen that the smaller basis set yields slightly better accuracy for both Slater and Dunning type basis sets. A similar phenomenon has been observed by Case³⁵ and coworkers when they compared the calculated NMR shielding parameters in peptides. Furthermore, the basis sets of Dunning-type producing chemical shifts agreed better with the experimental values than the Slater-type, which is not surprising because the former describes the core region electron density better. Better results were obtained at the PW1PW91/Aug-cc-pVDZ level, with the δ_{CRMSD} and δ_{HRMSD} values of 1.38 and 0.13 ppm, respectively, in accordance with the linear fitted equations (Fig. S2) between theoretical and experimental δ values.

The ^{13}C NMR signals were also calculated using the multi-standard approach (MSTD)⁴⁵ with the reference molecule of methanol. This method is likely to improve the agreement between the calculated and the experimental data as has been shown by Watts et al.⁴⁸ The δ_{CRMSD} obtained with the MSTD method is 1.25 ppm (Table 2), even better than that obtained at the same level with TMS as the reference molecule (1.38 ppm) while the δ value of hydrogen atom cannot be obtained with the MSTD method. Therefore, all the following calculations of β -cellobiose were carried out at the PW1PW91/Aug-cc-pVDZ level with the reference of TMS molecule.

3.2. Potential energy surface

The 2D potential energy surface (PES) contour of β -cellobiose is shown in Figure 2. Three energy minima are found, centered at the following ϕ/ψ torsion angles: $30^\circ/0^\circ$ (*syn- ϕ /syn- ψ*), $10^\circ/180^\circ$ (*syn- ϕ /anti- ψ*), and $180^\circ/5^\circ$ (*anti- ϕ /syn- ψ*) with *anti- ϕ /syn- ψ* being the lowest energy state. The minimum energy path demonstrates that the conversion from *syn- ϕ /syn- ψ* to the other two is facile owing to their low energy barriers ~ 8.0 kcal/mol (Fig. 2). This characteristic may attribute to the high flexibility of cellobiose. The direct transformation between *syn- ϕ /anti- ψ* and *anti- ϕ /syn- ψ* is obviously more difficult due to its higher barrier (over 20 kcal/mol) located at the ϕ/ψ angle of $120^\circ/-60^\circ$, where atoms O5', C1', O', and C4 are approximately in a plane and the distance between O3 and O5' is too short (1.9 Å). So the transformation between *anti- ϕ /syn- ψ* and *syn- ϕ /anti- ψ* more likely goes through *syn- ϕ /syn- ψ* .

Table 1¹³C and ¹H NMR parameters (ppm) for cellobiose obtained with various methods at the 6-311+G(d) basis set level

	1'	2'	3'	4'	5'	6'	1	2	3	4	5	6	RMSD ^c
¹³ C shift													
Exp. ^a	103.37	73.97	76.31	70.28	76.8	61.41	92.63	72.04	72.15	79.56	70.92	60.74 ^b	
B3LYP	110.13	78.58	84.38	74.95	82.95	65.48	96.56	74.7	75.1	79.43	75.6	60.87	6.11
M062X	108.90	77.88	83.43	74.04	81.28	64.97	98.35	78.50	78.54	86.78	77.26	65.51	
PW1PW91	106.50	75.07	80.60	71.19	79.40	62.66	102.8	81.99	81.42	86.65	81.01	65.34	4.93
WB97XD	106.72	75.70	80.86	72.02	79.60	62.89	96.30	77.27	76.90	85.88	75.47	65.02	
							100.71	81.34	79.75	85.39	79.67	64.92	2.70
							94.49	75.20	74.86	83.15	73.70	62.75	
							99.06	78.41	77.74	83.04	77.55	62.61	3.05
							94.28	75.40	75.57	83.73	74.04	63.23	
							99.11	78.84	78.24	83.62	77.73	63.03	
¹ H shift													
Exp. ^a	4.52	3.32	3.52	3.41	3.51	3.93	5.23	3.58	3.83	3.65	3.96	3.88	
B3LYP	4.73	3.74	3.60	3.93	3.33	4.02	4.67	3.29	3.63	3.63	3.59	3.96 3.81	0.24
M062X	4.56	3.74	3.43	3.79	3.12	4.19	5.55	3.84	3.96	3.86	4.05	4.32 4.03	
PW1PW91	4.65	3.67	3.51	3.85	3.25	3.94	4.83	3.40	3.83	3.88	3.61	4.24 4.08	0.24
WB97XD	4.51	3.60	3.57	3.84	3.22	3.92	5.38	3.53	3.90	3.82	3.95	4.42 3.85	
						3.93	4.67	3.19	3.82	3.92	3.52	4.38 3.92	0.19
						3.92	5.43	3.74	3.92	3.80	4.00	4.29 3.92	
						3.92	4.73	3.31	3.78	3.82	3.54	4.21 3.98	0.19
						3.92	5.39	3.70	3.83	3.68	3.93	4.37 3.89	
						3.90	4.78	3.27	3.68	3.68	3.47	4.31 3.93	

^a ±0.02 ppm, the data come from Ref. 58.^b The data of α and β form are collected in the first and the second line, respectively.^c RMSD refers the root mean square deviation of ¹³C or ¹H.**Table 2**¹H and ¹³C NMR parameters for cellobiose obtained with the PW1PW91 functional at different basis sets

PW1PW91	1'	2'	3'	4'	5'	6'	1	2	3	4	5	6	RMSD ^b
¹³ C shift													
6-311++G(d,p)	107.36	75.92	81.43	71.79	80.17	63.40	95.47	76.28	75.11	84.2	74.38	63.28 ^a	3.44
6-311++G(3df,3pd)	108.86	77.10	82.28	73.20	81.10	64.27	99.8	79.39	78.18	83.98	78.18	63.33	4.47
Aug-cc-pVDZ	103.26	71.82	76.39	68.10	76.33	59.31	96.99	77.15	76.53	85.02	74.70	64.33	
Aug-cc-pVTZ	107.48	75.23	79.74	70.86	79.81	63.13	100.95	80.89	79.86	84.51	78.64	64.28	1.38
MSTD ^c	103.42	72.08	77.34	68.13	77.00	59.47	90.73	72.68	71.07	80.26	70.11	59.48	
							94.90	75.40	74.13	80.39	73.23	59.58	3.22
							95.99	75.86	76.37	82.99	72.72	62.46	
							99.33	79.90	80.23	83.60	77.06	62.32	
							95.16	75.47	74.70	80.33	74.8	59.60	1.25
¹ H shift													
6-311++G(d,p)	4.49	3.54	3.45	3.70	3.23	3.93	5.31	3.67	3.82	3.67	3.94	4.23 3.84	0.15
6-311++G(3df,3pd)	4.53	3.44	3.44	3.73	3.35	4.00	4.55	3.23	3.63	3.71	3.44	4.14 3.89	
Aug-cc-pVDZ	4.45	3.43	3.43	3.69	3.34	3.97	5.35	3.63	3.79	3.66	3.92	4.23 3.80	0.14
Aug-cc-pVTZ	4.60	3.49	3.52	3.69	3.41	3.97	4.63	3.14	3.56	3.70	3.48	4.15 3.87	
						3.93	5.35	3.62	3.76	3.58	3.93	4.17 3.83	0.13
						3.97	4.57	3.12	3.56	3.61	3.53	4.12 3.87	
						3.96	5.36	3.69	3.85	3.70	3.97	4.22 3.83	0.13
							4.65	3.18	3.62	3.69	3.53	4.14 3.89	

^a The data of α and β form are collected in the first and the second line, respectively.^b RMSD refers the root mean square deviation of ¹³C or ¹H.^c MSTD means the multi-standard approach. The data are calculated with the reference of CH₃OH at PW1PW91/Aug-cc-pVDZ level.

After the reoptimization of the three stable states without the dihedral restraints, the ϕ/ψ dihedral angles of *syn-φ/syn-ψ*, *syn-φ/anti-ψ*, and *anti-φ/syn-ψ* change to 32.2°/−23.9°, 10.5°/180.0°, and 178.2°/−0.4° respectively. After the zero-point vibrational energy (ZPVE) correction, the energy difference ($\Delta E + \Delta ZPVZ$) between *syn-φ/syn-ψ* and *anti-φ/syn-ψ* is only 0.07 kcal/mol, suggesting that these two states are difficult to distinguish energetically. The Gibbs free energy (ΔG) of *syn-φ/syn-ψ* is 1.81 kcal/mol lower than *anti-φ/syn-ψ*. The ZPVE corrected energy and the Gibbs free energy of *syn-φ/anti-ψ* are higher than *anti-φ/syn-ψ* by 1.91 and 0.16 kcal/mol, respectively. The *syn-φ/syn-ψ* has the lowest Gibbs free energy (ΔG) in water (lower than *syn-φ/syn-ψ* and *syn-φ/syn-ψ* by ~1.8 and 2.0 kcal/mol), indicating that *syn-φ/syn-ψ* is the dominant conformation, consistent with the experimental findings.^{12,13,17,25} On the other hand, the

calculations clearly show that in water the free energy difference between these three states is small, considerably smaller than the theoretical results obtained in vacuum.^{40,49,50} In the gas phase, the relative energy of *syn-φ/syn-ψ* is higher than *anti-φ/syn-ψ* by 2.6 kcal/mol and lower than *syn-φ/anti-ψ* by 2.3 kcal/mol. While in water, the energy difference between *syn-φ/syn-ψ* and *anti-φ/syn-ψ* (*syn-φ/anti-ψ*) decreases to ~1.0 (1.8) kcal/mol (Table 3). Thus, the solvation effect decreases the energy differences of three cellobiose rotamers.

The structural analysis provides more insights about the relationship between the energy and the ϕ/ψ conformation. The effect of ϕ/ψ dihedral angle rotation on ring puckering is slight except for the distances of C1'O5' and C1'O', as shown in Table S1. The *anti-φ/syn-ψ* conformation has two intramolecular hydrogen bonds (O3H3...O2' (heavy atom distance of 2.812 Å, same below) and

Figure 2. Potential energy surface contour of β -cellobiose obtained at the PW1PW91/Aug-cc-pVDZ basis set level. The energy contours are at 1 kcal/mol intervals.

O6'H6'...O6 (2.888 Å)). The distance of H2' and H4 is 2.152 Å, shorter than the typical value of nonbonding H...H contacts (2.4 Å),⁶ suggesting the existence of the strong coupling between these two hydrogen atoms. The intramolecular O3H3...O5' hydrogen bond is observed in *syn-φ/syn-ψ* conformation, consistent with previous reports.^{25–27,51,52} The H...H repulsion is also found in this conformation between H1' and H4, with the distance of 2.123 Å, slightly shorter than that in the *anti-φ/syn-ψ* conformation. The *syn-φ/anti-ψ* conformation has a strong O3H3...O2' hydrogen bond (2.848 Å) and two pairs of the H...H repulsion, H1'...H3 (2.081 Å), and H1'...H5 (2.329 Å) respectively.

The structure and dynamics of hydrated cellobiose have been explored by means of ab initio dynamics.⁵³ Here, the solvent effect is examined with the explicit model for conformer *anti- ϕ /syn- ψ* . When the water molecule interacts with the hydroxyl groups at sites 1 and 2, the distances of O3H3...O2' and O6'H6'...O6 hydrogen bonds are 2.808 and 2.893 Å. The ϕ/ψ dihedral angle is 178.3°/−1.1°. These angles vary slightly (178.4°/−1.2°) when the water molecule couples with the hydroxyl groups at sites 3' and 4'. Correspondingly, the O3H3...O2' and O6'H6'...O6 hydrogen bonds are 2.796 and 2.895 Å, respectively. The effect of single water molecule on intramolecular hydrogen bonds and ϕ/ψ dihedral angles is slight.

It has been indicated the preferred energy structure varies when cellobiose dips into water from vacuum.⁵³ The initial structure of solvated cellobiose with large water cluster is conformer *anti- ϕ /syn- ψ* , which is surrounded by 52 water molecules. After optimization, the structure changes significantly (Fig. 3). The O3H...O2' interaction is substituted by the OH...O_W (2.636 and 2.738 Å) and O_WH...O (2.778 and 2.748 Å) hydrogen bonds. The

Figure 3. Optimized structure of hydrated cellobiose with 52 water molecules. Cellobiose is represented with the ball and bond model, while the water molecules are shown with the tube model.

O6'H6'...O6 coupling alters into the O6H...O6' (2.832 Å), with both oxygen atoms hydrogen bonded with water molecule. Therefore, the solvation effect of large water cluster on intramolecular hydrogen bonds and ϕ/ψ dihedral angles is prominent.

3.3. IR spectra

The structural (especially the hydrogen bond) variation can be reflected in the IR spectra. Distinct shift in wavenumber can be observed upon environmental perturbation, hydrogen bond emergence, or disappearance. Figure 4 indicates that the characteristic vibration of the O3H...O5' stretching mode locates at 3646.1 cm^{-1} in *syn-φ/syn-ψ*. While in *syn-φ/anti-ψ*, the vibration at 3649.3 cm^{-1} is assigned as the O3H...O2' stretching mode. This mode can also be observed in *anti-φ/syn-ψ* structure, where the other hydrogen bond stretching mode is found at 3645.5 cm^{-1} .

The hydration effect on IR spectra of the hydrogen bond is slight. It is inspected for conformer *anti-φ/syn-ψ*. When water is combined with the hydroxyl groups at 1 and 2 sites, the O3H...O2' and O3H...O5' vibrational modes stretch at 3620.1 and 3647.2 cm⁻¹, respectively. These two modes locate at 3609.9 and 3647.8 cm⁻¹ as the water molecule binds with the hydroxyl groups at 3' and 4' sites. Therefore, *anti-φ/syn-ψ* can be identified conveniently because it has two hydrogen bond stretching modes around 3600 cm⁻¹. But, it is difficult to distinct the *syn-φ/syn-ψ* and *syn-φ/anti-ψ* conformations with the IR spectra.

Table 3
Relative energy of three cellobiose conformations in the gas phase and water

	Gas phase			Water		
	$syn-\phi/syn-\psi$	$anti-\phi/syn-\psi$	$syn-\phi/anti-\psi$	$syn-\phi/syn-\psi$	$anti-\phi/syn-\psi$	$syn-\phi/anti-\psi$
ϕ^a	34.1	178.1	12.5	32.2	178.2	10.5
ψ	−16.9	−1.2	179.9	−23.9	−0.4	180.0
ΔE^b	2.60	0.00	4.93	0.96	0.00	2.80
$\Delta E + Z$	1.62	0.00	4.04	0.07	0.00	1.91
ΔG	−0.32	0.00	2.52	−1.81	0.00	0.16

^a ϕ and ψ are in °; the unit of the energy is kcal/mol.

^b ΔE means the relative energy; $\Delta E + Z$ corresponds to the zero-point corrected energy; ΔG refers the Gibbs free energy. The relative energies are obtained according to that of *anti- ϕ /syn- ψ* in the gas phase and water, respectively.

Figure 4. IR spectra of three local minima structures.

3.4. NMR chemical shifts

The variations of chemical shift as the function of ϕ and ψ were investigated and those of C1' and C4 showed a strong conformational dependence (Fig. 5). The chemical shifts of C1' ($\delta_{C1'}$) are 103.3 (*syn-φ/syn-ψ*), 98.9 (*anti-φ/syn-ψ*), and 95.3 (*syn-φ/anti-ψ*) ppm, respectively. For C4, the corresponding chemical shifts (δ_{C4}) are 80.4 (*syn-φ/syn-ψ*), 70.0 (*anti-φ/syn-ψ*), and 75.6 (*syn-φ/anti-ψ*) ppm, respectively. Correspondingly, the experimental data for $\delta_{C1'}$ and δ_{C4} of *syn-φ/syn-ψ* are 103.4 and 79.4, respectively. The error is only 0.1 ppm for $\delta_{C1'}$ and 1.0 ppm for δ_{C4} . Therefore, the error is negligible as compared with the chemical shift variation between different conformations. Once the δ values of the characteristic positions are determined, the conformer can be confirmed.

When ϕ ranges between -90° and 90° , the variation of ψ has a significant effect on $\delta_{C1'}$, which can change from 93.2 (*syn-φ/anti-ψ*) to 107 (*syn-φ/syn-ψ*) ppm. In contrast, the $\delta_{C1'}$ value alters less when $\phi < -90^\circ$ or $\phi > 90^\circ$. From the geometric viewpoint, C1' and H4 are in *cis*-conformation while $-90^\circ < \phi < 90^\circ$. The influence of ψ rotation on $\delta_{C1'}$ is significant probably owing to the steric effect between C1' and H4 which is covalently bonded to C4. On the contrary, C1' and H4 are in the *trans*-conformation when $\phi < -90^\circ$ or $\phi > 90^\circ$. Correspondingly, the variation of $\delta_{C1'}$ is slight in the absence of such a steric effect between C1' and H4.

A similar phenomenon can be observed for the δ of C4 presented in Figure 5b. The influence of ϕ on δ_{C4} is distinct when $-90^\circ < \psi < 90^\circ$. The amplitude of change is about 20.0 ppm. The steric repulsion between C1' and H4 varies along the rotation of ϕ and potentially contributes to the alteration of $\delta_{C1'}$. Another observation from Figure 5b is that δ_C changes dramatically from ~ 63 to ~ 83 ppm with ψ rotation from *anti-φ/syn-ψ* to *syn-φ/*

syn-ψ when ϕ is around $\pm 120^\circ$. In this geometry, C4 locates on the plane determined by O5'C1'O'C2'. A significant steric effect occurs when the dihedral angle ψ varies from the *cis* ($-90^\circ < \psi < 90^\circ$) to the *trans* ($\psi < -90^\circ$ or $\psi > 90^\circ$), corresponding to the transfer from *syn-φ/anti-ψ* to *anti-φ/syn-ψ*. As is consistent with the analysis about PES that the transfer between *syn-φ/anti-ψ* and *anti-φ/syn-ψ* is difficult owing to the high energy barrier.

The H1' and H4 chemical shifts (δ_H) variations as the function of ϕ and ψ are about 2 ppm (Fig. S3). The experimental data for $\delta_{H1'}$ and δ_{H4} of *syn-φ/syn-ψ* are 4.52 and 3.63 ppm, respectively. The theoretical value at the PW1PW91/Aug-cc-pVDZ level for $\delta_{H1'}$ and δ_{H4} of *syn-φ/syn-ψ* are 4.45 and 3.61 ppm, respectively, in good agreement with the experimental data. Similar to that of C1', the $\delta_{H1'}$ changes distinctly along with ψ when $-90^\circ < \phi < 90^\circ$ but alters slightly when ϕ is out of this range. For δ_{H4} , it varies mainly along with ϕ when ψ ranges from -90° to 90° . When ϕ approaches $\pm 120^\circ$, distinct variations can also be observed with the changes of ψ , similar to that of C4.

The microenvironmental effect is investigated with the explicit model for monohydrated conformer *anti-φ/syn-ψ*. When the hydration occurs at site 1 and 2, the $\delta_{C1'}$, δ_{C4} , $\delta_{H1'}$, and δ_{H4} are 98.9, 70.0, 4.8, and 4.0 ppm, respectively. They are equal to the values obtained with the implicit model for conformer *anti-φ/syn-ψ*. These data shifts are undetectable to 98.6, 69.9, 4.8, and 4.0, respectively, as the water molecule binds at 3' and 4' site. Therefore, the effect of hydration on C1' and C4 is slight.

The effect of chemical environment (such as solvent, temperature, and magnetic field) as well as the error of functional and basis set on δ can be removed, at least to some degree, when the chemical shift difference is considered. The advantage of using the chemical shift difference is the chemical shift reference elimination. Figure S4 presents the values of chemical shift difference of both C1'–C4' (plot a, $\delta_{C1'-C4'}$) and C1–C4 (plot b, δ_{C1-C4}). When the difference of $\delta_{C1'-C4'}$ is about 25.0 ppm, the conformation locates in the *syn-φ/anti-ψ* region (Fig. S4a). The ϕ and ψ dihedrals are in the *syn-φ/syn-ψ* area when $\delta_{C1'-C4'}$ is about 39.0 ppm. The *anti-φ/syn-ψ* region can be identified by the value of δ_{C1-C4} if it is about 30.0 ppm (Fig. S4b).

A conformational population corresponds to a specific value of $\delta_{C1'-C4'}$ and δ_{C1-C4} . The values of $\delta_{C1'-C4'}$ and δ_{C1-C4} in various conformations are shown in Figure S5. Plot (a) shows distinctly that the *syn-φ/syn-ψ* conformation occupies 100% of the total population when the $\delta_{C1'-C4'}$ is 39.7 ppm. Following the population decreasing of *syn-φ/syn-ψ* conformer, $\delta_{C1'-C4'}$ reduces and δ_{C1-C4} increases simultaneously. The population of *syn-φ/anti-ψ* and *anti-φ/syn-ψ* rises during this process, as can be drawn from both a and b plots. The conformation should change completely to *syn-φ/anti-ψ* when $\delta_{C1'-C4'}$ and δ_{C1-C4} are 24.5 and 22.7 ppm,

Figure 5. 2D contour plot for chemical shifts of C1' (a) and C4 (b) atoms.

Table 4
 ^{13}C shielding of C1' and C4 in β -cellobiose at optimized conformations

ϕ ($^\circ$)	ψ ($^\circ$)	C1'						C4					
		Principal components (ppm)		Direction cosines ^a				Principal components (ppm)		Direction cosines ^b			
				x	y	z				x	y	z	
32.2	−23.9	σ_{11}	73.9	−0.1048	−0.5025	0.8582	σ_{11}	89.9	−0.0959	0.8557	0.5084		
		σ_{22}	91.1	−0.2637	0.8461	0.4633	σ_{22}	97.6	−0.0582	−0.5147	0.8554		
		σ_{33}	107.4	0.9589	0.1777	0.2212	σ_{33}	153.6	0.9937	0.0525	0.0992		
178.2	−0.4	σ_{11}	76.4	0.0841	−0.3057	0.9484	σ_{11}	94.9	0.1825	−0.0242	−0.9829		
		σ_{22}	102.1	0.6400	−0.7130	−0.2866	σ_{22}	123.7	−0.0732	−0.9973	0.0110		
		σ_{33}	111.5	−0.7638	−0.6310	−0.1357	σ_{33}	152.4	0.9805	−0.0699	0.1838		
10.5	180	σ_{11}	74.1	−0.1340	−0.3056	0.9427	σ_{11}	93.7	−0.1083	−0.0657	−0.9919		
		σ_{22}	102.3	0.8720	−0.4883	−0.0343	σ_{22}	110.8	−0.0323	−0.9971	0.0695		
		σ_{33}	123.7	−0.4708	−0.8174	−0.3319	σ_{33}	155.9	0.9936	−0.0396	−0.1059		

^a The x-axis is along the C1'–O' bond, and the xz-plane is defined by the H1'C1'O' group.

^b The x-axis is along the C4–O' bond, and xz-plane is defined by the H4C4O' group.

Figure 6. Scheme for the shielding component direction in three conformations. For C1', the x-axis is along the C1'–O' bond and the z-axis is in the H1'C1'O' plane. The x-axis of C4 is along the C4–O' bond and the xz-plane is defined by the H4C4O' group.

respectively, (Fig. S5a). While, if all conformation alters to *anti-phi/syn-psi*, the $\delta_{\text{C1}'-\text{C4}'}$ and $\delta_{\text{C1}-\text{C4}}$ are detected at 32.0 and 31.2 ppm, respectively (Fig. S5b).

Our calculations show clearly that C1' and C4 chemical shifts are sensitive to (ϕ, ψ) values, and thus can be useful in distinguishing the oligosaccharide conformations. For example, it has been discovered that ionic liquids (ILs) are good solvents for cellulose and the cellulose conformation in ILs has been of a great interest. A recent theoretical study⁵⁴ shows that the IL changes the conformation of polysaccharide (1–4 linked β -D glucose) as compared with that in water solution. But the carbon NMR spectra of cellulose and cello-oligomers are shown to be similar in aqueous and ionic liquid (IL) solutions, although their physical and chemical properties are extremely different.^{55–57} Based on our calculation, its (ϕ, ψ) conformation should be *syn-phi/syn-psi*, same as in solution.

3.5. Shielding tensor

Table 4 collects the shielding results for C1' and C4 in three energy minimum conformations: *syn-phi/syn-psi* ($\phi = 32.2^\circ$, $\psi = -23.9^\circ$), *anti-phi/syn-psi* ($\phi = 178.2^\circ$, $\psi = -0.4^\circ$), and *syn-phi/anti-psi* ($\phi = 10.5^\circ$, $\psi \sim 180^\circ$). For the C1' shielding tensor, a molecular frame is defined, which has the x-axis lying along the C1'–O' bond and the z-axis being on the H1'C1'O' plane, to give a better view about the eigenvectors. The direction cosines of three eigenvectors are given with respect to the molecular frame (Table 4). Figure 6 shows the directions of the shielding components at the three conformations with a schematic drawing. The least shielded component (σ_{11}) of C1' lies approximately on the yz plane and tilted toward z-axis. The variation of this component is small for both the shielding eigenvalue and eigenvector. However, σ_{22} changes more, from 91.1 ppm (*syn-phi/syn-psi*) to 102.1 ppm (*anti-phi/syn-psi*) and 102.3 ppm (*syn-phi/anti-psi*) whereas the corresponding eigenvector rotates clockwise (around σ_{11} vector which is approximately

unchanged) by 150° (*syn-phi/syn-psi* to *anti-phi/syn-psi*) and 130° (*syn-phi/syn-psi* to *syn-phi/anti-psi*), respectively. The most shielded component (σ_{33}) of C1', lying along the C1'O' bond in *syn-phi/syn-psi*, also has a dramatic variation, from 107.4 ppm (*syn-phi/syn-psi*) to 111.5 ppm (*anti-phi/syn-psi*) and 123.7 ppm (*syn-phi/anti-psi*). Thus, the effect of ϕ and ψ rotation to C1' is mainly on σ_{22} and σ_{33} .

The most shielded component (σ_{33}) and its eigenvector of C4, lying roughly along the x-axis defined by the C4–O' bond vector, remain approximately unchanged for these three conformations. Along the conformational changes of ϕ (ψ) from *syn-phi/syn-psi* to *anti-phi/syn-psi*, two components (σ_{11} and σ_{22}) rotate by $\sim 60^\circ$ (Fig. 6). Furthermore, the eigenvector of σ_{11} (as well as σ_{22}) remains unchanged in *anti-phi/syn-psi* and *syn-phi/anti-psi*. σ_{22} has the largest eigenvalue variations, from 97.6 ppm (*syn-phi/syn-psi*) to 123.7 ppm (*anti-phi/syn-psi*) and 110.8 ppm (*syn-phi/anti-psi*).

4. Conclusion

In summary, the potential energy surface of β -cellobiose in water was constructed as the function of the H1'C1'O/C4 (ϕ) and C1'O/C4H4 (ψ) dihedral angles at the PW1PW91/Aug-cc-pVDZ level. Three energy minima were identified, corresponding to *syn-phi/syn-psi*, *anti-phi/syn-psi*, and *syn-phi/anti-psi*. It is found that the hydrogen bonding and the H...H repulsion are vital for the energy of cellobiose. The solvation effect of large water molecular cluster on cellobiose structure is prominent. The C4 (C1') chemical shift (δ) is sensitive to the conformational changes, with the values of 80.4 (103.3) at *syn-phi/syn-psi*, 70.0 (98.9) at *anti-phi/syn-psi*, and 75.6 (95.3) at *syn-phi/anti-psi*. The error is negligible as compared with the chemical shift variation between different conformations. The chemical shielding tensor analysis shows that the directions and amplitudes of two components (σ_{22} and σ_{33}) of C1' shielding tensor vary distinctly with the ϕ and ψ . In comparison, σ_{33} of C4 remains unchanged, but σ_{22} , varies by 13 (26) ppm from *syn-phi/syn-psi* to *anti-phi/syn-psi* (*syn-phi/anti-psi*).

Acknowledgments

This work was supported by 100 Talent Project, the Knowledge Innovation Program of the CAS (Grant No. KSCX2-EW-J-10), National Nature Science Foundation of China (Grant Nos. 21173247, 31270785 and 21203227), the Foundation for Outstanding Young Scientist in Shandong Province (No. BS2010NJ020), the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry, and the Research Foundation for Talented Scholars of the Qingdao Agricultural University (No. 631335).

Supplementary data

Supplementary data (the optimized parameters of three minima, the correlation between calculated and experimental chemical shift, the 2D contour plots for chemical shifts of H1' and H4, the 2D contour plots for the chemical shift difference of C1' and C4' ($\delta_{C1'-C4}'$) as well as C1 and C4 (δ_{C1-C4}), and the value of $\delta_{C1'-C4}'$ and δ_{C1-C4} in various conformers) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.carres.2014.12.006>. These data include MOL files and InChIKeys of the most important compounds described in this article.

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