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Structure and conformation of α-, β- and γ-cyclodextrin in solution: Theoretical approaches and experimental validation

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ABSTRACT

The anomeric carbon chemical shifts of α-, β- and γ-cyclodextrin in solution were studied experimentally and theoretically by NMR and two-layer ONIOM2 (B3LYP/6-31G*-GIAO: HF/6-31G*-GIAO) variant. The dependence between the anomeric carbon chemical shift and the glycosidic bond φ and ψ dihedral angles in D-Glcp-D-Glcp disaccharides with and (1 → 4) linkages in α-configurations were computed by Gauge-Including Atomic Orbital (GIAO) ab initio and ONIOM in water solvent using PCM methods. Complete chemical shift surfaces versus φ and ψ for this disaccharide were computed. We also present empirical formulas of the form $^{13}C\ \delta = f(\varphi,\psi)$ obtained by fitting the ab initio data to trigonometric series expansions. The results are consistent with experimental observations and show the applicability of chemical shift surfaces in the conformational behavior of oligosaccharides.

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1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides built up from α-D-glucopyranose units connected with a α-1,4 glycosidic linkage. The CD molecule comprise 6 glucopyranose units, while β- and γ-CD comprise 7 and 8 units, respectively, arranged in a ring-shape manner and presenting a conical shape, Fig. 1. They have ability to form hydrogen bonds with the surrounding water molecules or intermolecular ones (Hesek, Hembury, Drew, Borovkov, & Inoue, 2001) and inclusion complexes with a large variety of organic and inorganic guests (Prabaharan & Mano, 2006; Uekama, 2002).

One of the most remarkable applications of CDs is its use as drug carriers in controlled release systems. For example as drug carriers, β-CD allows the solubilization, stabilization, and transport of hydrophobic drugs together with several pharmacological benefits such as the reduction of unwanted side effects (Hedges, 1998; Uekama, Hirayama, & Irie, 1998). During the complex formation with drug molecules, no covalent bonds exist between the CD and its guest, thus complexation can be considered as a dynamic process. The drug molecules included within the CD cavity therefore may be dissociated upon dilution, displaced by a more suitable guest, or transferred to a matrix for which it has a higher affinity, such as a biological membrane (Loftsson & Brewster, 1996). Therefor reveal the structural details of cyclodextrin could be so benefit.

Between different methods to study of carbohydrate especially oligosaccharide conformation, theoretical calculation of NMR properties, in combination with NMR experimental data, can produce lots of information about the structure details of such molecules (Adeyeye et al., 2003; Brien & Moyana, 2004; Wormald et al., 2002). In most of these approaches, estimation of internuclear distances between oligosaccharide proton pairs is obtained using 1D or 2D nuclear Overhausser enhancement (NOE) experiments, and structural information is derived from homo and hetero nuclear vicinal (^3J) and geminal (^2J) coupling constants.

Carbohydrate ^{13}C chemical shifts have been employed as another valuable source of structural information that could be used instead of NOE and *J*-coupling data. In particular, the anomeric carbon chemical shift is depended on the glycosidic bond; φ and ψ dihedral angles (Jarvis, 1994).

There are many uses for applying computational chemistry to the study of CD, especially in the structural characterization of host–guest complexes by ab initio (Dobado & Benkadour, 2004) molecular mechanics (MM) (Momany & Willett, 2000) molecular dynamics (MD) (Choi, Yang, Kim, & Jung, 2000), and semiempirical methods (PM3, MNDO AM1, etc.), (Lipkowitz, 1998). To our knowledge, due to the size of these systems quantum-mechanical calculations consist of DFT and ONIOM (Svensson et al., 1996) methods on CDs including solvent effect have not been reported. Thus, the aim of the present work is to evaluate the applicability of these computational approaches in characterizing the structural details and predicting the NMR chemical shifts in α-, β- and γ-CDs by comparison with experimental data.

As pointed out in a recent review by Imberty and Pérez (2000) the conformational dependence of chemical shifts in oligosaccharides has been poorly understood. Since in this report we examined

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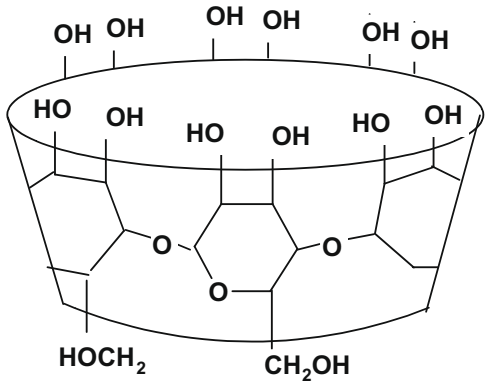

Fig. 1. Structures of the cyclomaltooligosaccharides α-CD ($n = 6$), β-CD ($n = 7$) and (c): γ-CD ($n = 8$).

applicability of ^{13}C chemical shift to study the carbohydrate conformation, using the surface corresponding to the D-Glcp α (1 → 4) linkage with α-, β- and γ-cyclodextrin as model systems. The results show that ^{13}C data obtained from theoretical chemical shift surfaces reproduce experimental observation in solution and serve as a useful model.

Therefore we present structural (geometries) and spectroscopic (NMR chemical shifts) characterizations for a series of modified α-, β- and γ-cyclodextrin, that can help synthetic chemists in assigning of the NMR spectra of the CD derivatives and inclusion complexes.

2. NMR measurements

^{1}H NMR, ^{13}C NMR, COSY, HMQC and HMBC of α-, β- and γ-CD were obtained at 298 K in D_2O (99.99% D) on a Bruker DRX500 operating at 500.133 MHz for ^{1}H and 125.770 MHz for ^{13}C, using 5 mm broad band inverse probe with sufficient digital resolution. Sodium 4,4-dimethyl-4-silia-[2H4] pentanoate was used as internal standard. ^{1}H NMR and ^{13}C NMR spectra were acquired using a spectral width of 3255 and 22,123 Hz, respectively, and a 90° pulse (10.3 μs). All 2D NMR spectra were acquired by pulsed field gradient-selected methods. 2D correlation spectroscopy (COSY) was used to confirm ^{1}H assignments. Heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC) were used for ^{13}C assignments, Fig. 2. HMQC and

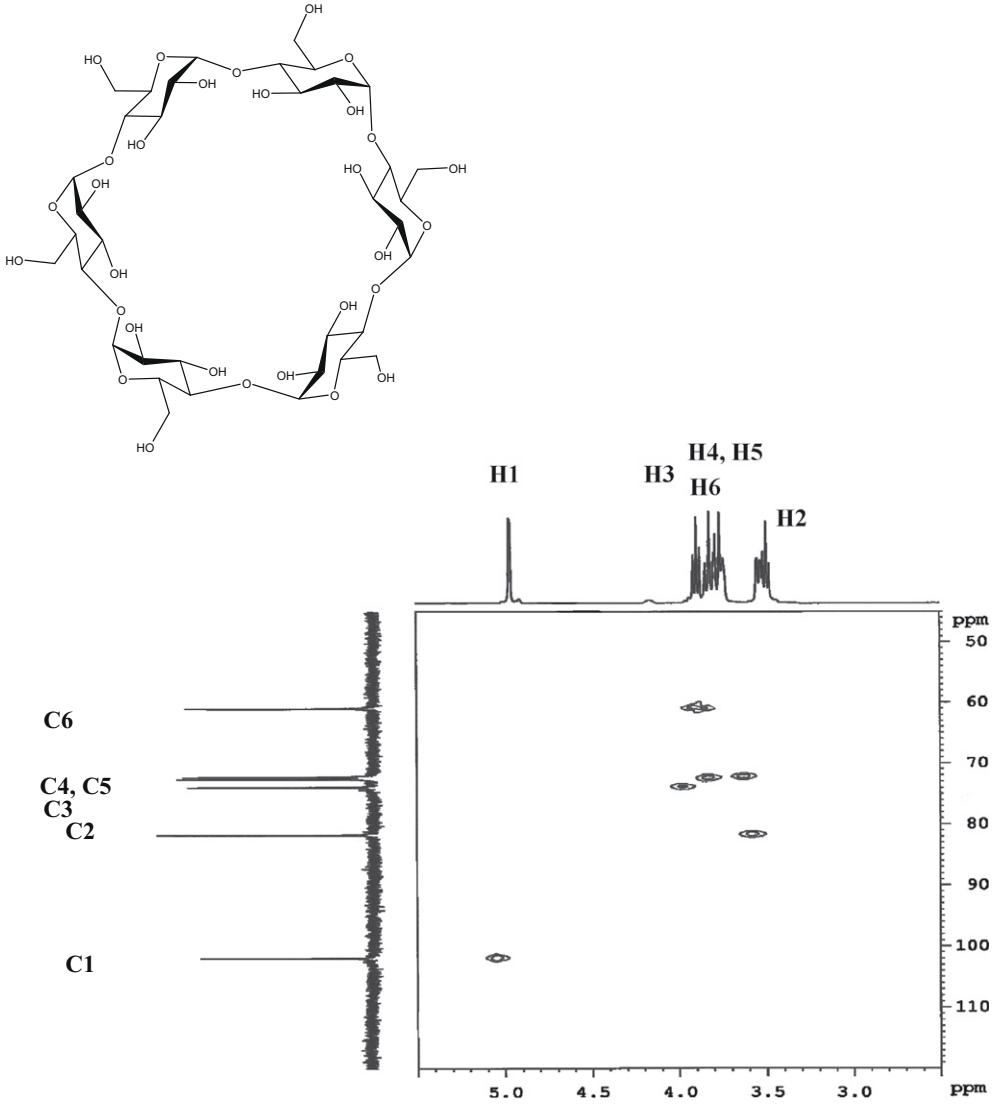

Fig. 2. HMQC spectrum of α-cyclodextrin.

HMBC spectra were recorded using 2048 × 1024 data matrices; the number of scan and dummy scans were 48 and 16, respectively, in all cases. The spectral width in COSY experiments with pre-saturation during relaxation delay was 3255 Hz in both dimensions with 2 s relaxations delay. The HMQC and HMBC spectra were recorded with 2 s inter pulse delay. The spectral widths were: $sw_1 \times sw_2 = 3255 \times 22{,}123$ Hz in all 2D experiments. For Z-only gradients, the G1:G2:G3 = 50:30:40.1 gradient ratios were used for both HMQC and HMBC spectra.

3. Computational details

All calculations were carried out with the Gaussian program series 2003, as basic program and HYPER CHEM version 7.0 and Gaussian Viewer as graphical medium. Full geometry optimization of all molecules is performed at the program default conditions without any symmetry constrain imposed with FOPT keywords with C1 point group. Two levels of calculations, HF/6-31G* and ONIOM (our own *n*-layer integrated molecular orbital and molecular mechanics), have been used for calculations. The ONIOM approach subdivides a molecule into several parts or layers, each of which is described data at different level of theory. Subsequent layers are treated using progressively computationally cheaper lower-level approaches; in this study we use the two layers system, ONIOM2 (B3LYP/6-31G*: HF/6-31G*).

The solvent effects on the conformational equilibrium have been investigated using the PCM method for water as a solvent ($\varepsilon = 78.39$).

The Gauge-Including Atomic Orbital (GIAO) method as implemented in Gaussian 2003 was employed for all NMR calculations (Wolinski & Pulay, 1990) and by employing the density functional theory (DFT) has accurately predicted the ^{13}C chemical shifts (García-Granados et al., 2000, 2003). In order to verify the performance of the ONIOM approach for the calculation on NMR shielding constants, the ONIOM2 (B3LYP/6-31G*-GIAO: HF/6-31G*-GIAO) scheme was applied to the calculation of the absolute isotropic NMR shielding in the water.

4. Results and discussion

4.1. Geometry optimization and energies of α-, β- and γ-cyclodextrin

Molecular structure for α-, β- and γ-CD, were built from their corresponding X-ray coordinates (Betzel, Saenger, Hingerty, & Brown, 1984; Harata, 1987; Manor & Saenger, 1974). Gaussian viewer were used to convert CSD coordinate files in to PDB files and add hydrogen in idealized positions in structures. In first step we fully optimized these structures at HF/6-31G* level of theory in the gas phase, then the out put of this calculation was used as an input for optimization with ONIOM method. Two-layer ONIOM scheme, in which the higher level of theory is B3LYP/6-31G*, including all atoms in pyranosid ring, and the lower level of the theory is HF/6-31G*, including all hydrogen atoms, hydroxyl and exocyclic hydroxymethyl group, Fig. 3.

Since the complexity of the conformational equilibrium is believed to a combination of several factors such as solvent effects, therefore the next step in study was to include conformational averaging effects in solution to estimate chemical shifts. Since the polar hydroxyl groups in these molecules interact strongly with water and form extensive H-binding. This interaction can alter the conformational space accessible to the CDs in solution (Lipkowitz, 1998). The previous study (Tafazzoli & Ghiasi, 2007) on monosaccharide shows the PCM model is suitable for solvent effect. So all structures in water solvent ($\varepsilon = 78.39$) using polarized continuum (overlapping spheres) model (PCM), of Miertus and Tomasi (1982), were reoptimized. The result shows all compounds in water are about 7 kcal/mol more stable than the gas phase. Fig. 4 shows the optimized structures of α-, β- and γ-CD in water. Selection of calculated bond distances, bond angles, and dihedral angles are compiled in Table 1.

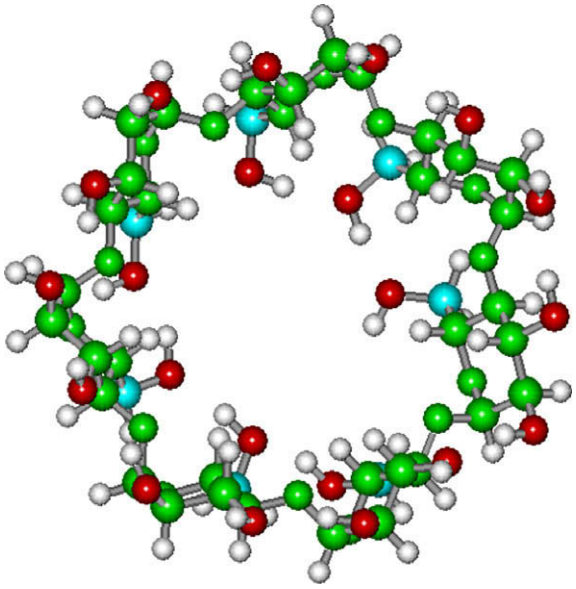

Fig. 3. Presentation of two layers system of β-CD was used for ONIOM method, the green atoms in the inner layer were optimized with B3LYP/6-31G* method and the other atoms in outer layer were optimized with HF/6-31G* method. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

In order to explore the applicability of ^{13}C chemical shift surface in study of cyclodextrin conformation, we need to calculate the ^{13}C chemical shift surface for the disaccharide model, including (1 → 4) glycosidic linkages in α-configuration. So in the next step we optimized the disaccharide model to calculate the ^{13}C chemical shift.

4.2. Generation of the D-glocopyranosis-α-(1-4)-D-glocopyranosis chemical shift surface

D-Glcp-D-Glcp disaccharide models, including (1 → 4) glycosidic linkages in α-configuration, were built with HYPERCHEM 7. In order to generate the chemical shift surfaces as a function of the glycosidic bond conformation, an 18 × 18 grid in the $-180° \leftrightarrow 180°$ range for φ and ψ at 20° intervals was constructed, to make a total of 324 input structures for model molecule. For each structure in the grid, the φ and ψ were kept constant with dihedral constraints on heavy atoms and the monosaccharides held in the chair conformation, while the rest of the molecule was optimized using the B3LYP/6-31G* method. To consider the solvent effect the PCM method was employed and optimized D-Glcp-D-Glcp disaccharide models in water were used as input for ab initio ^{13}C chemical shift shielding calculations, Fig. 5.

4.3. Isotropic ^{13}C chemical shift of disaccharide model and fitting of raw ab initio data

In order to obtain the ^{13}C chemical shifts surface, the GIAO ^{13}C calculations using the B3LY/6-31G* method were performed on fully optimized structures of D-Glcp-α-(1 → 4)-D-Glcp linkages in gas phase and in the solution. For comparing these results with results of calculated ^{13}C chemical shifts of cyclodextrin, the ONIOM2 (B3LYP/6-31G*-GIAO: HF/6-31G*-GIAO) was utilized to calculate ^{13}C chemical shifts of disaccharide model, Fig. 6. In all cases, the isotropic ^{13}C chemical shift was estimated by subtracting the iso-

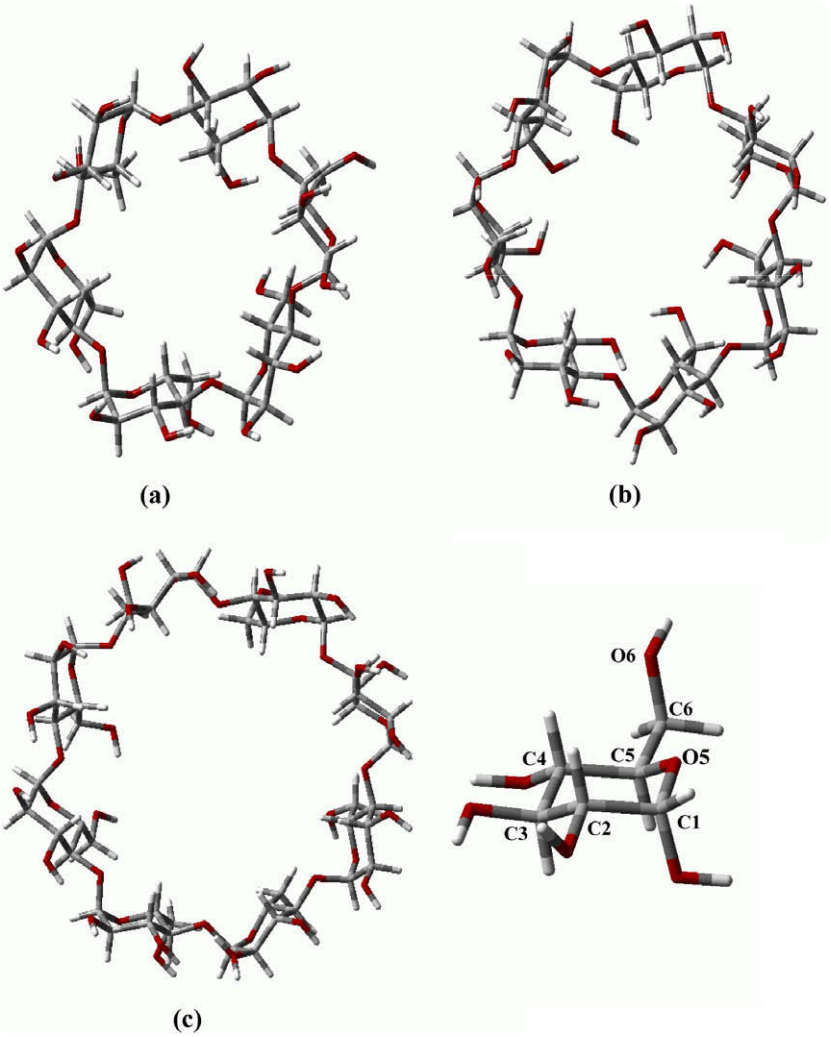

Fig. 4. Optimized structures of (a): α-CD, (b): β-CD and (c): γ-CD in water using ONIOM method.

Table 1
Some of optimized structural details of α-, β- and γ-CD in water and the gas phase (parentheses) by ONIOM method.

	α-CD	β-CD	γ-CD
Bond distance (Å)			
C1O5	1.43(1.42)	1.42(1.41)	1.39(1.41)
C1C2	1.50(1.50)	1.52(1.51)	1.51(1.52)
C2C3	1.51(1.52)	1.51(1.50)	1.51(1.51)
C3C4	1.52(1.53)	1.51(1.51)	1.52(1.52)
C4C5	1.52(1.52)	1.52(1.52)	1.53(1.52)
C5C6	1.51(1.52)	1.53(1.52)	1.52(1.53)
Bond angles (°)			
C1O1C4	110.58(110.70)	113.88(112.23)	116.09(114.27)
C1C2C3	111.19(110.85)	109.77(110.13)	110.07(111.21)
C5C6O6	108.51(109.12)	110.97(111.42)	110.04(110.13)
Dihedral angles (°)			
O1C1C2C3	53.42(53.68)	56.15(55.65)	47.10(51.12)
O1C5C4C3	−57.24(−58.12)	−60.86(−61.58)	−60.54(−61.23)

tropic chemical shielding of the carbon atom to the one obtained at the same theory level for the methyl carbons of the NMR reference tetramethylsilane (TMS). For consistency, the geometry of TMS was also optimized using the B3LYP/6-31G* in ^{13}C chemical shift computations.

The obtained isotropic ^{13}C shifts for anomeric carbons were employed in the derivation of empirical equations relating the glycosidic bond φ and ψ dihedral angles to the ^{13}C chemical shift. The calculated B3lyp/6-31G* ab initio chemical shift data were fitted to trigonometric series expansions of general form:

$$^{13}C\delta_{\text{anom}}(\varphi,\psi) = \sum_i [A_i \sin(i\varphi) + B_i \cos(i\varphi) + C_i \sin(i\psi) + D_i \times \cos(i\psi)] + \sum_{i,j,\alpha,\beta} [A_{i,j,\alpha,\beta} \sin(i\alpha)\cos(j\beta) + B_{i,j,\alpha,\beta} \times \sin(i\alpha)\sin(j\beta) + C_{i,j,\alpha,\beta}\cos(i\alpha)\cos(j\beta)] + C_0 \quad (1)$$

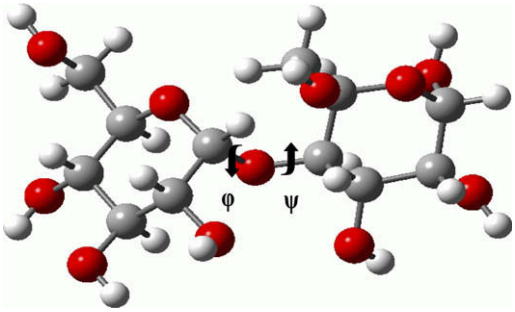

Fig. 5. Optimized structure of D-Glcp-D-Glcp disaccharides with (1 → 4) linkages in α-configurations in water using ONIOM method.

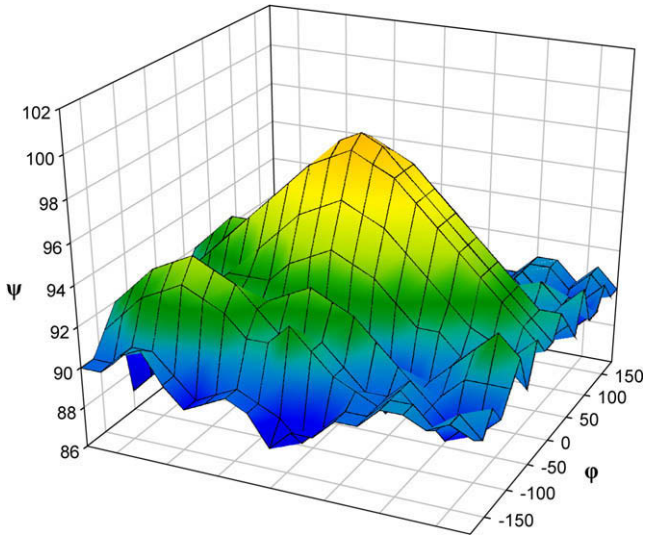

Fig. 6. ^{13}C chemical shift surfaces for the anomeric carbon of D-Glcp-α-(1 → 4)-D-Glcp obtained using ONIOM method in solution.

Table 2
Comparison of calculated and experimental anomeric carbon chemical shifts (ppm) for α-, β- and γ-cyclodextrin in solution by ONIOM method.

Molecules	Calculated chemical shifts (ppm)	Experimental chemical shifts (ppm)
α-CD	102.12	102.55
β-CD	103.22	103.00
γ-CD	103.17	102.40

where α and β can be either φ or ψ. The data were fitted using Mathematica 3.0 (Wolfram Research, Inc.), using a series with 91 terms (i, j = 1–3).

4.4. Chemical shift of CD structures in solution

In order to investigate the applicability of ^{13}C chemical shift surfaces to the study of oligosaccharide conformation and dynamics, their accuracy need to be determined first. This is hindered by the fact that most of the available NMR data for carbohydrates are in solution, therefore the measurements are affected by conformational averaging. These systems provide us the chance to compute anomeric carbon chemical shifts using the D-Glcp-α-(1 → 4)-D-Glcp chemical shift surface in order to compare the theoretical estimations directly against experimental ^{13}C chemical shift data. The optimized structures of α-, β- and γ-CDs in water was used to calculate ^{13}C and ^{1}H chemical shifts using ONIOM method. The NMR spectroscopic data are tabulated in Table 2. These data consist of the calculated ^{13}C chemical shifts, with the experimental data. The computed ^{13}C chemical shifts correlate well with experimental results, particularly in comparison of mean values for the three molecules. The results obtained for α-CD are more informative. The torsional strain caused by its smaller ring size forces D-Glcp residues 1 and 2 in this cyclomaltooligosaccharides to deviate from a regular ring shape arrangement, resulting in glycosidic linkage angles for the 1–2 and 2–3 monomer pairs of (φ = −32.5, ψ = −5.5) and (φ = −31.5, ψ = −52.5), respectively. The calculated ^{13}C chemical shifts for the anomeric carbons of these two residues are 102.70 and 98.58 ppm, respectively, which are in very good agreement with the observed ^{13}C chemical shifts of 102.10 and 98.10 ppm.

5. Conclusions

In this report, we applied ab initio and ONIOM methods in order to show the dependence of the anomeric carbon chemical shift to the glycosidic bond dihedral angles for a D-Glcp-D-Glcp disaccharide models, including (1 → 4) glycosidic linkages in α-configuration. Our results, which are in good agreement with experimental observations and earlier theoretical calculations, indicate that the relationship between the chemical shift of the anomeric carbon and the φ and ψ dihedral angles can be conveniently represented by the function of ^{13}C $\delta(\varphi,\psi)$. Also we presented the application of chemical shift surface derived from GIAO ONIOM calculations to study structure of α-, β- and γ-CD. The results show that this method is capable of predicting the anomeric carbon chemical shifts of cyclomaltooligosaccharides in solution.

In summary, the chemical shift of anomeric center is affected by the glycosidic bond torsion and the corresponding chemical shift surfaces would constitute independent but complementary indicators of linkage conformation. Therefore the ^{13}C chemical shift surface is a suitable method to predict experimental anomeric carbon chemical shifts in the study of oligosaccharide conformation. The advantage of this method is the measurement of ^{13}C chemical shifts is easier than measurement of *J*-coupling and NOE correlation. Furthermore, ^{13}C resonances are not affected by signal overlap as seriously as *J*-coupling, NOE correlations and other NMR parameters which are dependent on ^{1}H chemical shifts. Thus both experimental chemical shifts and ^{13}C shift surface can provide important data about the oligosaccharides structure where ^{1}H chemical shifts overlap makes the measurement of other NMR parameters difficult. Also current study shows, the integrated multi layer ONIOM approach makes a promising debut in the theoretical NMR area. As a result of the division of the molecular system in two layers which can be described at different levels of theory, with the highest level reserved for the most important part of the molecule only, ONIOM achieves considerable computational saving over conventional approaches which treat the entire molecular system at a uniform level of theory.

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