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REGULAR ARTICLE

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Carbohydrates and quantum chemistry: how useful is this combination?

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Abstract In this work three illustrative situations are reported of how quantum mechanics can be successfully employed to describe important properties of carbohydrates, a very large and versatile class of compounds found in nature. A proper quantified description of the main stereoelectronic effects that determine the behaviour of such molecules in the biological medium is offered by quantum mechanics better than any other theoretical approach. Such statement is supported by the conformational work discussed for disaccharides, by the rate coefficients found in aqueous solution for the glucose mutarotation process and still for the optical rotation values found for each conformer of glucose in aqueous solution.

Keywords Carbohydrate · Conformational map · Disaccharide · Optical rotation · Mutaroration

1 Introduction

Carbohydrates are a very important class of compounds widespread in all realms in nature. Their biological importance is related to many different processes in living organisms as energy production and storage, molecular recognition, defense and structure [1]. Examples of molecules that belong to this class and are involved in the cited processes are glucose, glycogen and starch, glycoproteins, imunoglobulins and cellulose. As they are very versatile molecules with many different applications, it is easy to understand why they belong to a multidisciplinary research area, where people having different backgrounds such as organic chemists, engineers, biologists and pharmacists are working. Since the background of the researcher automatically defines their approach, the study of carbohydrates has different approaches.

One possible approach seeks to obtain the structure of the compound at an atomistic level. The relevance of this study can be immediately understood when the two systems composed of only glucose are considered in Fig. 1.

In the first case, the types of the bonds between the monosaccharidic units are α-1,4 and α-1,6, and in the second case it is only β-1,4. While glycogen is used as energy store in animals, cellulose has structural functions in plants. Thus, the kind of glycosidic bond defines structure that mainly determines function [2]. Many other examples could be cited, but may be sufficient to recall that all carbohydrates present in the human body are *dextro* molecules, while the *levo* partners are not biologically useful. The conformational difference between the *dextro* and the *levo* structures is large enough to confer on each enanciomer remarkable biological differences. Therefore, in order to better understand any process where a carbohydrate molecule is involved at a mechanistic level, it would help very much if its structure and also its conformation were known.

Nevertheless, obtaining the conformation of a carbohydrate at an atomistic level is not a simple task, and much has been done in both theoretical and experimental fields. The difficulties of obtaining such conformation are due to the fact that carbohydrates are poly-functionalised compounds, whose groups are similar and interact very intensively each other. Therefore, in the experimental framework they generate spectra whose assignment peaks are often difficult, and in the theoretical framework, they have potential energy surfaces full of minima with very similar depth. And it is exactly at this crossroad that the scope of this paper is better understood. It would be useful to describe the main interactions present in these molecules which are responsible for their conformation, as best as possible under the atomistic point of view, and the quantum mechanics is excellently suited to do that better than any other theoretical approach.

On the other hand, recent years have witnessed a huge development of computers and computational codes. Until some years ago, to perform calculations on large systems as simple as a monosaccharide was not a feasible task. However, more efficient computers and codes became available and now we can perform routinely ab initio calculations on molecules as large as disaccharides in reasonable time. The time

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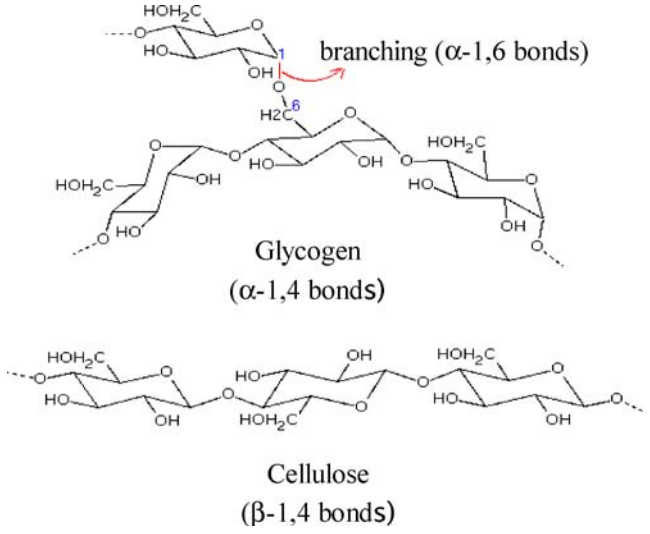

Fig. 1 The type of the glycosidic bond between the monomeric units defines the three-dimensional structures of cellulose and glycogen

therefore is perhaps ripe for applying also quantum mechanics to gain information about systems of biological interest whose access is not possible by other approaches.

Based on these ideas, some examples of how quantum mechanics can bring more light to the discussions about carbohydrate chemistry will be presented in the next three sections entitled: conformational aspects, chemical reactions and properties of carbohydrates.

2 Conformational aspects

2.1 General remarks

Surely the large majority of the papers found in the literature devoted to conformational studies of carbohydrates theoretically oriented are based on classical methods [3–15], and the reason for this is the size of the molecules. Clearly, the classical methods as molecular mechanics and dynamics are very useful, principally for systems that cannot be treated yet by quantum methods. However it is important to keep in mind that when a theoretical approach is chosen to access the structure/conformation of any particular compound, it is mandatory to assure that such theoretical model properly describes the main stabilizing effects present in such a structure. The most important stabilizing effects in carbohydrates are the hydrogen bonds between the hydroxyl groups, the non-bounded interactions that comprise electrostatic and van der Waals interactions [16], and the anomeric effect [17]. As presented better by the chemiometric studies of Pérez et al. [18] and Engelsen and coworkers [19], these effects are very difficult to be parameterised into generic or even specific second generation force fields, quoting the authors, there is ample room for development of new third generation carbohydrate force fields, based on quantum mechanical calculations. In their work they compared twenty different force fields in their capacities of describing the energy difference between the α and β anomers for the glucopyranose molecule, in other words, in their capacities of properly quantifying the anomeric effect, and also in their capacities of quantifying the stabilization energy that comes out from a hydrogen bond. The results reported by the authors show that the large variation for such energy differences, for both effects studied, can range up to 10 kcal/mol depending on the force field. Therefore, perhaps, a non-parameterized theoretical description could be useful, and we have decided to try a full ab initio description to study conformations in carbohydrates.

2.2 Methodology

Some years ago, the relaxed and rigid residue approaches used to build conformational maps of disaccharides using molecular mechanics were compared [20]. The results were interesting, but they are not immediately transferable to a quantum mechanical context. The immediate convenience of such transferring is the computational time saved by the use of rigid conformational maps instead of the relaxed ones.

In order to check the possibility of replacing relaxed conformational maps by the rigid ones, both approximations were tested for β-D-galactopyranosyl-(1→4)-β-D-glucopyranose, referred to hereafter as $\beta-$lactose, in the gas-phase. The starting geometry was obtained from molecular mechanics using Dreiding II force field [21]. This structure was fully optimised at HF/6-31G(d, p) [22] level and the dihedral angles that define the glycosidic bond (ϕ and ψ) are shown in Figure 2. The hydroxymethyl orientations are GG for both moieties. A Hartree–Fock (HF) conformational map was calculated in the following way: ϕ and ψ angles were scanned in 12 steps of 30° each. The respective 144 structures obtained by all combinations had their respective energy values calculated by two different approaches [20].

In the first approach, called rigid residue method, single point calculations were performed to each value of ϕ and ψ,

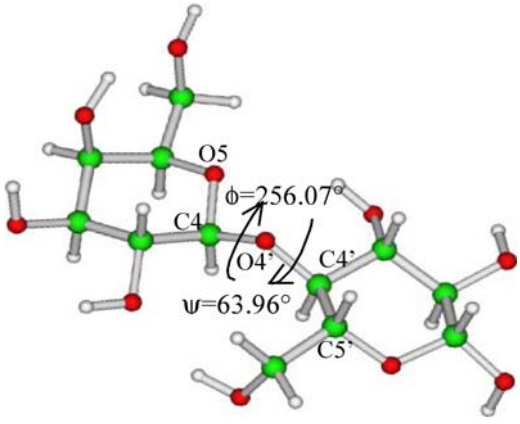

Fig. 2 $\beta-$lactose in gas phase, in the GGGG conformation of the hydroxymethyl groups. The values of the dihedral angles $\phi$and ψ (defined respectively by the sequence O5-C1-O4′- C4′ and C1-O4′-C4′-C5′), establish the orientation of the glycosidic linkage

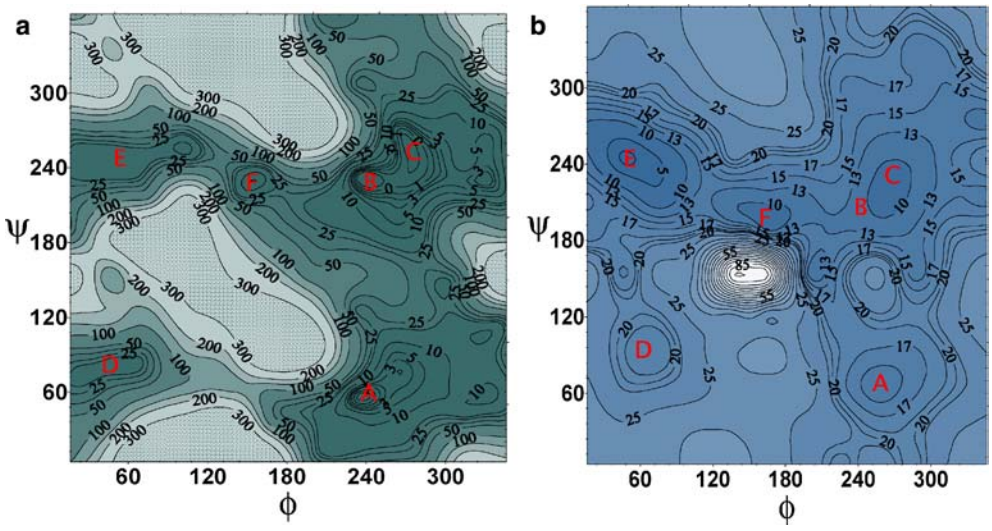

Fig. 3 Rigid (**a**) and relaxed (**b**) conformational maps calculated for $\beta-$lactose at HF/6-31G(d, p) level (144 points each map), in the gas-phase. The energy contour values are in kcal/mol

while in the second approach the molecule was allowed to optimise its geometry to the new ϕ and ψ angles, at each step of the scanning process.

2.3 Results and discussion

The conformational maps obtained from both approaches are shown in Fig. 3.

The dotted regions in Fig. 3a are regions of high energy where excessively hindered structures that come out sometimes from rigid rotations are found. These regions cannot be described in the rigid residue approach, since such congested structures had to be eliminated. Due to this procedure, there is no information available about such regions from these maps. It is important to recall that each structure in this approximation is obtained from rigid rotations of ϕ and ψ, and no geometrical adaptation of the molecule to these new ϕ and ψ values is allowed.

Comparing the relaxed map with this rigid one, the stability regions are found in the same locations, regardless of the approximation used. This coincidence may be an indication how strong are the interactions that define the glycosidic angles, since they can still be described by the rigid residue approach. Another important point that must be stressed is the fact that in none of the two approaches can we identify any global minimum. Due to the scanning procedure, even in the relaxed approach, two degrees of freedom are still kept frozen, namely the ϕ and ψ angles on which the scanning is performed. The term "global minimum" in a rigorous sense must be applied only when all degrees of freedom are optimised. Six stability regions are found in both maps, and named as A, B, C, D, E and F [23].

The same procedure used to generate the conformational map for β-1,4 lactose in the gas-phase, was repeated within the rigid residue approach, but this time introducing the solute–solvent interaction potential into the hamiltonian. Doing this, the solvent effects on the solute are taken into account following the IEFPCM [24,25] version of the polarizable continuum model (PCM) [26,27]. The same HF/6-31G(d, p) calculation level adopted in the gas-phase was used in aqueous solution, and the initial structure was obtained from full optimisation of the equivalent starting structure used to generate the maps in the gas-phase. More details about the calculations performed is regard to the solvent and the anomeric effects can be found elsewhere. [23,28,29].

In Fig. 4, the rigid conformational map obtained for β-lactose, considering the solvent effects through a dielectric continuum model is reported.

As can be seen from comparison of Figs. 3 and 4, the regions of stability found in the map that contains the solvent effects on the disaccharide are the same of those found for the disaccharide in the gas-phase. No different regions of stability were found in the map that contains the solvent effects that were not present in the map in the gas phase, where only intramolecular interactions are present.

The six previous conformers that were fully optimised comprised the glycosidic angles, and the F structure converged to the E structure during this process. After introducing thermal and entropic corrections as well as the solvent effects, the respective populations were obtained in the gas-phase and in solution, and two of these structures (B with $\phi = 263°$ and $\psi = 218°$ and C with $\phi = 283°$ and $\psi = 235°$) were found with representative populations in the gas phase and aqueous solution at 298 K. Starting from these two structures, all the GT and GG orientations possible for the hydroxymethyl groups were investigated. The (TG) trans gauche conformations were not taking into account since they are not so abundant as the GG and GT ones [8,30].

Full geometrical optimisations were carried out, entropic and thermal effects introduced, and solvent effects considered as described elsewhere [23]. The populations obtained

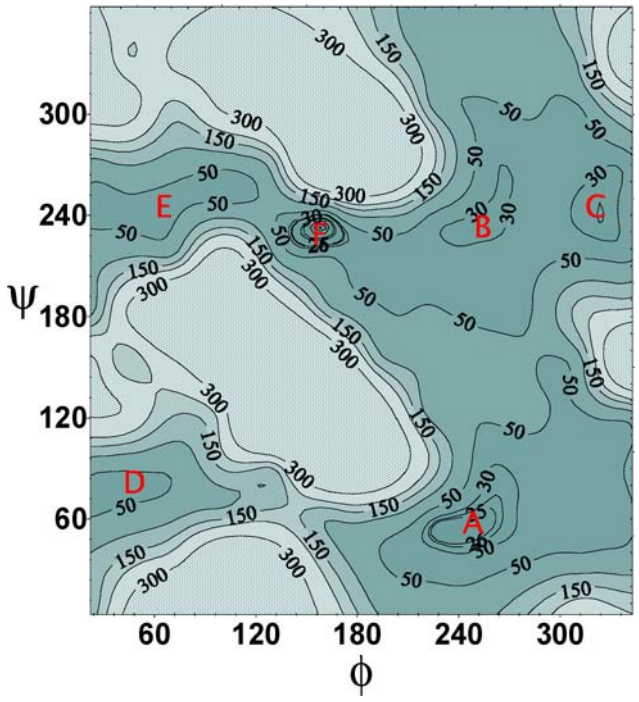

Fig. 4 Rigid conformational map calculated for β-lactose at HF/6-31G(d, p) level, in aqueous solution. Energy contour values are in kcal/mol

in solution are reported in Table 1. The GTgalGGglu for the conformers B and C converged to the same final structure. From Table 1, it can be seen how a rotation on the hydroxymethyl group influences on the glycosidic angles.

In order to validate these conformers, the $^{3}J_{H,C}$ (heteronuclear spin coupling constant) for the atoms along the glycosidic bond was calculated for each structure by an empirical expression [31], and its average weighted by the Boltzmann population. Table 2 shows the comparison between the experimental and calculated values obtained from this procedure. Other structures, obtained from conformational maps calculated using classical methods are also tested. The agreement between the $^{3}J_{H,C}$ values found from the structures selected in this work and the experimental ones can be considered as satisfactory, principally if the number of conformers is taken into account.

Table 1 Most abundant structures in aqueous solution, found for β-lactose

Structure	ϕ	ψ	%Pop.^{solution}
GGgalGGglu B	263°	218°	8.00
GTgalGGglu B/C	260°	260°	11.89
GGgalGTglu B	281°	228°	21.84
GTgalGTglu B	267°	203°	32.75
GGgalGGglu C	283°	235°	5.00
GGgalGTglu C	280°	227°	9.37
GTgalGTglu C	266°	202°	10.91

The angles are in degrees. All the quantities related to these calculations can be found elsewhere [23]

Table 2 Calculated and experimental $^{3}J_{H,C}$ values for the atoms along the glicosidic linkage in β−lactose, methyl β−lactoside and ethyl β−lactoside

	This work[a]	Exp.[b]	Exp.[c]	MM3[c,d]	PEF953[e,f]	PEF95G[e,g]
H1, C4′	4.13	3.8	3.7	3.5	3.8	3.6
H4′, C1	4.30	4.9	–	4.2	4.1	3.4

[a] Seven conformers
[b] Ref. [32]
[c] Ref. [7]
[d] Based on a Boltzmann distribution at 300 K from ΔE, using 34,443 conformations
[e] Ref. [10]
[f] Calculated from a Boltzmann distribution at 300 K from DE, using 24,925 conformations
[g] Calculated from a Boltzmann distribution at 300 K from DG, using five conformations

At this point, from the available data, it seems to be possible to find reliable conformations for disaccharides in aqueous solution from rigid maps calculated in the gas-phase, followed by a dielectric continuum description of the solvent effects that basically affects the energetics of the the system.

This procedure was extended to other disaccharides and the confomational maps for cellobiose and trehalose was obtained from both rigid and relaxed approaches. The validation of the conformers found are in progress, as also the investigation of the solvent effects on both maps. Besides, it would be interesting to apply this quantum mechanical methodology to obtain conformational maps for some systems that present different minima in the gas-phase and in aqueous solution, when a classical description is adopted [15].

Finally, it is important to mention that ab initio calculations in aqueous solution, within the PCM approach, are more time consuming than calculations in the gas-phase, and relaxed conformational maps in aqueous solution are not yet feasible for structures as large as disaccharides. Due to this, it is important to know how much information is lost, when the rigid map is adopted instead of the relaxed one to describe the torsional effects of the glycosidic bond of a disaccharide in aqueous solution [29]. In other words, the possibility of replacing relaxed conformational maps by the rigid ones is a procedure that deserves more investigation.

3 Chemical reactions

3.1 General remarks

Basically more than 99% of the glucose occurrence in aqueous solution is in the pyranosidic form [2]. Through the generation of a hemiacetal compound, the so-called mutarotation process leads to the occurrence of monosaccharides in this pyranosidic form. It is responsible for the interconversion between their α and β cyclic anomers, passing through an open structure, as shown in Fig. 5.

While in aprotic solvents the proton transfer is intramolecular, in aqueous solution there is experimental evidence that some solvent molecules may assist it. In specialized

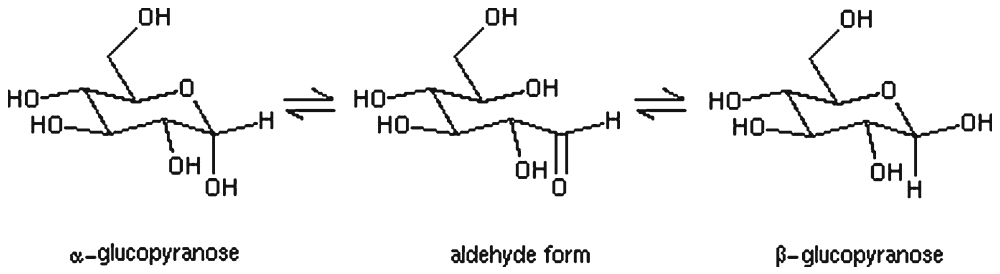

Fig. 5 Mutarotation of α and β forms of glucose

textbooks [2], although the vapor pressure of carbohydrates is very low at room temperature signifying that the amount of the substance in the gas phase is negligible, the mutarotation is always explained as a general intramolecular process and no solvent is considered. Nevertheless, it is important to keep in mind that if the situation to be considered in any model is as close as possible to the biological media, the solvent here is a part of the process that cannot be forgotten or disregarded.

Due to its biological importance, the mutarotation of D-glucose has been extensively studied experimentally through the years [33–49].

Regarding a theoretical approach to the glucose mutarotation, recently Yamabe and Ishikawa [50] performed a quantum mechanical study in aqueous solution using the Onsager model [51], assisted by a few water molecules. They concluded that two or three water molecules may assist this reaction through a strain-free hydrogen-bond network for a ready proton transfer. The reaction barrier for the pyranose ring opening is strongly reduced by this water assistance. The barrier height diminishes from 50.84 kcal/mol in an intramolecular proton transfer, to 24.88 kcal/mol when three water molecules assist the reaction. However, in their work, these authors have used in all calculations the α and β forms of TG glucose conformer, which is not the most abundant conformer of glucose in aqueous solution [52–57].

Considering the discussion presented in the preceding paragraphs, in the next paragraphs we will present a quantum mechanical study of the mechanism of glucose mutarotation in aqueous solution, considering the most abundant conformer of glucose, as well as a much more sophisticated continuum model to describe the bulk solvent effects [24–26]. We will take into account the behavior of this reaction when the proposed mechanisms do and do not consider water molecule assistance in aqueous solution.

Besides, from the data obtained, we shall try to estimate the rate coefficient of this process using just theoretical calculations in order to better compare the reaction pathways studied with the available experimental data, this, we belive, in the first time it has been done.

3.2 Methodology

The pyranose and aldehyde forms of glucose used in this work are the α and β forms of the GT conformer, for which the dihedral angle ω= O5-C5-C6-O6 is approximately gauche (60°), as reported in Fig. 6. These are the most abundant conformers found in aqueous solution, about 66% as reported in the literature [52–57]. The other α and β GG rotamers ($\omega = -60°$) are less abundant in aqueous solution (approximately 21%). However, as it cannot be considered a negligible population, some considerations will also be outlined about its contribution to the mutarotation process. The α and β conformers inter convert each other through an open form, whose abundance in solution is negligible.

All the structures studied were determined at the B3LYP [58,59] level of calculation, using the 6–31+G(d, p) basis set. Considerations about the use of density functional calculations and this basis set are better presented in the work of Csonka [22]. All the stationary points on the potential energy surface in the gas phase or in aqueous solution, corresponding to either a minimun or a transition structure, were characterized by the calculation of the respective vibrational frequencies. These calculations were performed using the Gaussian 03 program [60]. In order to define the role of the solvent in the mutarotation of glucose, we will study two descriptions to this reaction, as indicated below.

Model 1: Continuum solvent The system is studied in aqueous solution with a dielectric continuum description for the solvent. In this model we do not include any specific solute–solvent interaction. The proton transferring is intramolecular, and the solvent does not take part assisting it. There are just bulk electrostatic effects collaborating to the stabilization of all structures.

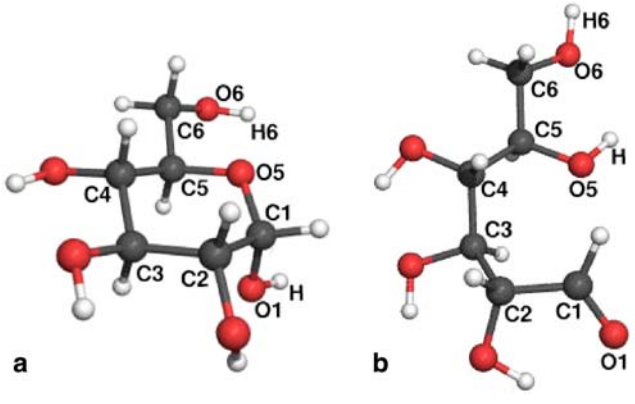

Fig. 6 pyranose (**a**) and aldehyde (**b**) forms of α-glucose. The atom labeling follows the standard adopted for carbohydrates

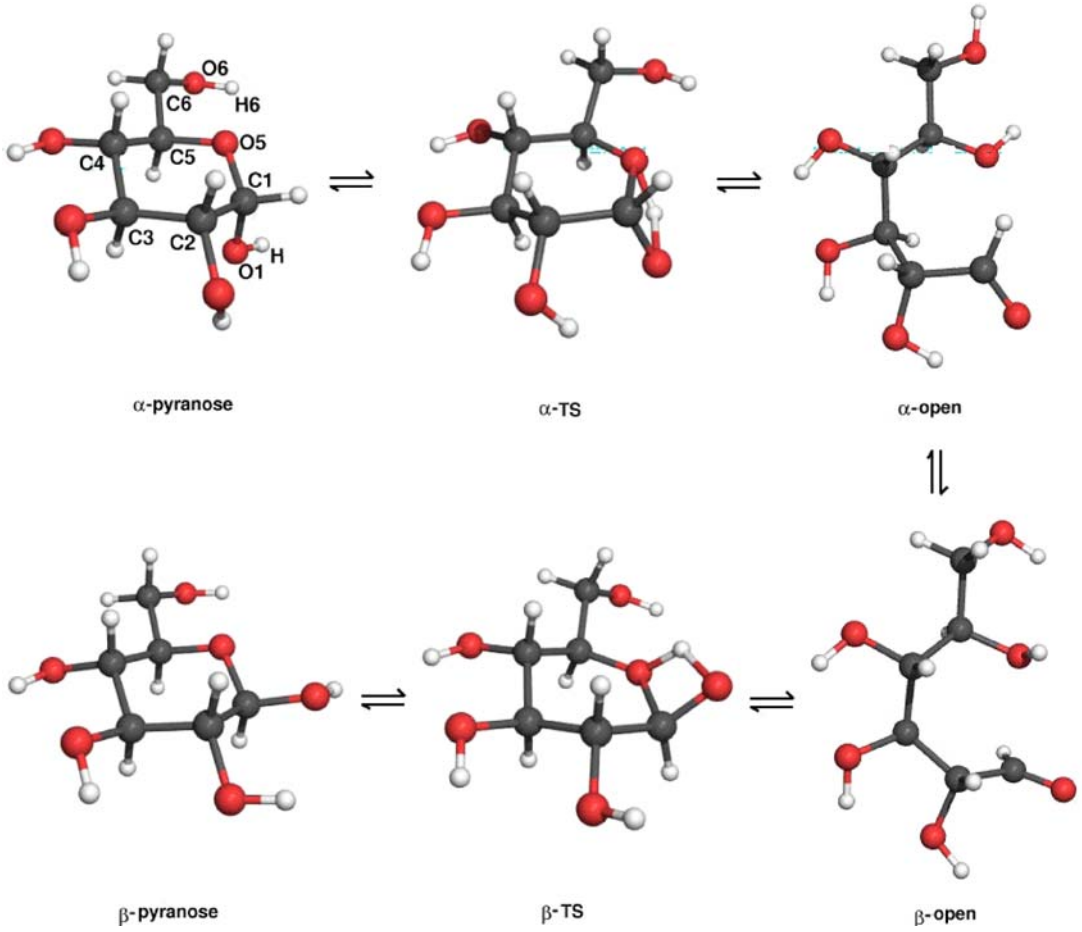

Fig. 7 The concerted mechanism of mutarotation of glucose, as described from Model 1

Model 2: Microsolvation and continuum The cluster consisting of glucose plus one water molecule is studied in a polarizable dieletric continuum media, to make possible the analyses of both effects, the specific interactions coupled to the bulk effects. The proton transfer is intermolecular, meaning explicitely solvent assisted.

Geometry optimization calculations were carried out to obtain the structures of reactants, products, and saddle points. After this, as already mentioned, their vibrational frequencies were obtained in order to check whether the stationary points were correctly located, i.e., if there were just positive frequencies for reactants and products, and only one negative frequency for transition state related to the bond disrupted. The intrinsic reaction coordinate has been followed, in some cases, to observe the evolution from the transition states to reactant and products, principally in the case of Model 2 where the potential energy surface is very shallow, with a large density of minima. The effects of solvation were determined using the integral equation formalism [25] version of the solvation model [26].

3.3 Results and discussion

The two pathways investigated are reported in Figs. 7 and 8, where the intramolecular and intermolecular mechanisms are considered, respectively. The structures exhibited are those obtained from the ab initio description characterized by the corresponding frequency calculations.

Comparing Figs. 7 and 8, the ring distortion of the TS structures in Model 1 is evident, and needed to the intramolecular proton transferring process. Clearly such deformation happens at the expenses of an increase in the energy of the system, when both processes are compared. All the main modifications in the lengths, angles and torsional angles of these structures are presented and discussed in detail in the complete work (A.M. Silva et al. Submitted).

Figure 9 shows the variation of the Gibbs free energy in the aqueous solution of all structures studied for Models 1 and 2 considering thermal and entropic corrections.

From Fig. 9 the diminishing of the height of the barrier for the glucose mutarotation when the solvent does and does not assist it is striking. In the first case (Model 2) there is no distortion of the ring of the TS structures, since the proton transfer is assisted by a solvent molecule in a concerted mechanism. A solvent molecule helps the proton transfer, exchanging one proton with the glucose molecule (Fig. 8). The values found for the height of the barriers are 26.32 kcal/mol for the α-pyr$\rightarrow$ α-TS transition and 23.93 kcal/mol for the β-pyr$\rightarrow$ β-TS transition for the GT rotamer. Only Model 2 was applied to the GG rotamer, and although less abundant than the GT

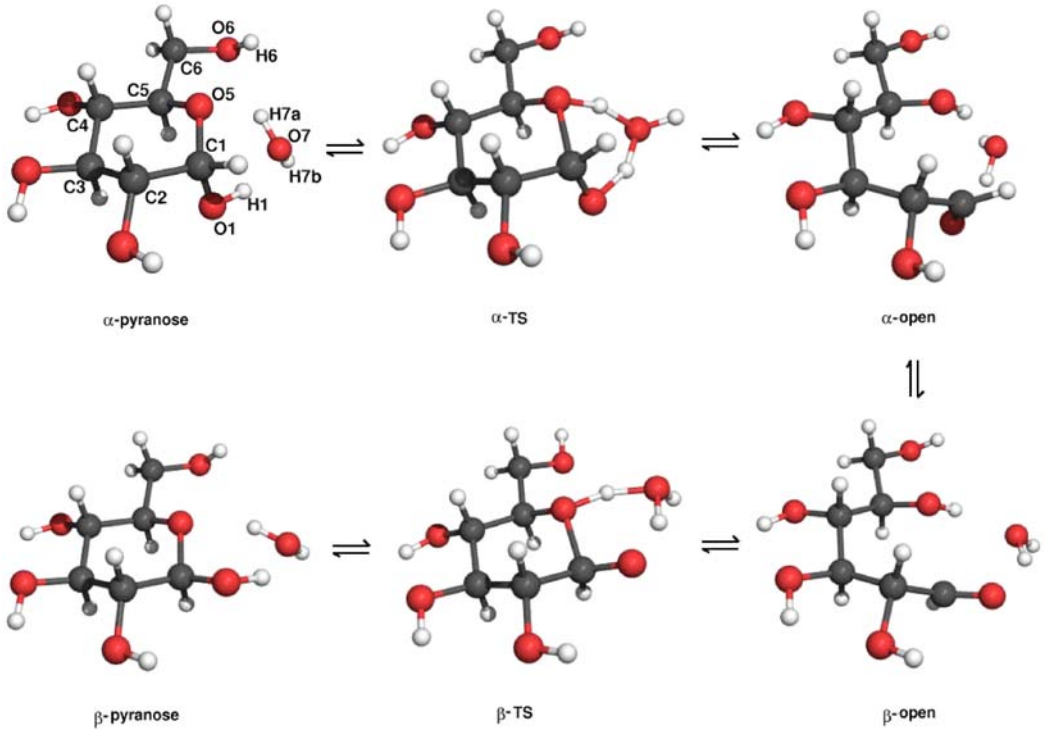

Fig. 8 The concerted mechanism of glucose mutarotation, as described from Model 2

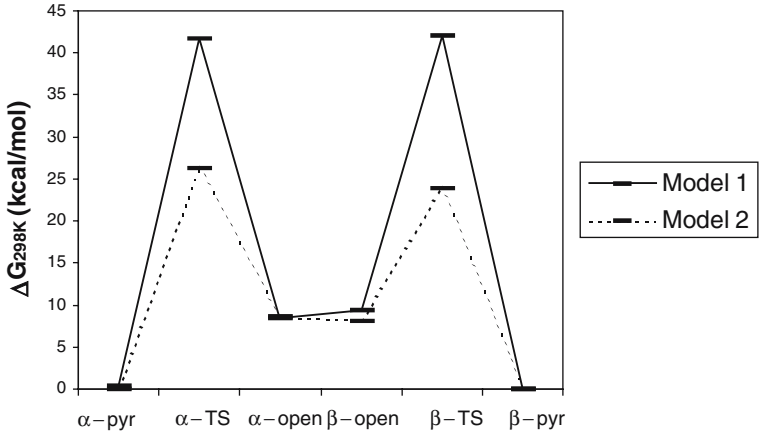

Fig. 9 The profile of the Gibbs free energy variation for the mutarotation reaction in Models 1 and 2, in kcal/mol. G_{298K} (β−pyr) = −687.098830 a.u. in Model 1 and −763.526751 a.u. in Model 2), for GT conformer

structure, the mutarotation process seems to be favored in the former. The height of the barriers are 23.68 kcal/mol for the α-pyr→ α-TS transition and 23.11 kcal/mol for the β-pyr→ β-TS transition. Adopting here the *effective free energy* concept [61], which applies the Arrhenius formula assuming that the rate constants of the individual channels can be summed to give the total rate constant, the new values for the barrieries are 23.68 kcal/mol for the α-pyr→ α-TS transition and 22.92 kcal/mol for the β-pyr→ β-TS transition, considering *both* GT and GG conformers.

In order to check the reliability of the mechanisms studied, the rate coefficients of each step of the mutarotation were theoretically obtained using the transition state reaction rate theory [62,63]. It is based on the application of statistical mechanics to reactants and the corresponding activated complex (TS). Within this context, the rate coefficient (k) for the glucose mutarotation is given by Eq. (1) as

$$k = \frac{k_B T}{h} N_A \frac{Q^{\neq}}{Q_A} e^{-(\Delta E^{/}_{act}=)/RT} \quad (1)$$

where h, N_A, k_B and R are Planck, Avogadro, Boltzmann and ideal gases constants, respectively. T is the temperature of the system, E_{act} is the activation barrier which includes already the zero-point energy correction. In the equation, $Q^{\neq}$ and Q_A

are the partition functions for the transition state and for the reactant A, respectively. Each partition function is written as the product $Q = q_{rot} \cdot q_{vib} \cdot q_{eletr} \cdot q_{trans}$, where the first two components q_{rot} and q_{vib} are the rotational and vibrational partition functions obtained from the second derivatives of the energy regarding the nuclear coordinates, q_{eletr} is equal to 1 (only one electronic state is considered), and q_{trans} is assumed to be similar for reactants and transition structures, hence cancelled in the Eq. (1).

In the gas phase, this approach is immediate once the second derivatives for each stationary point corresponding to each structure involved in the mechanism are calculated. However, in aqueous solution, to the best of our knowledge, this is the first time that this approach is tested, assuming a dielectric continuum description for the solvent. In this case, the tacit assumptions are: (1) the partition function in solution can be factorized and written as a product of the partition function for the solute (glucose in the case of Model 1, and glucose plus 1 water molecule in the case of Model 2) and the partition function of the solvent, (2) the partition function for the solvent is approximately the same for the transition structure and for the reactant, thus, both terms are cancelled in the expression, retaining just the Q for the solute (TS and reactant), directly obtained from the second derivatives of the PCM model. In this case, the $\Delta E^{\neq}_{act}$ is replaced by the corresponding quantity in solution, meaning, the Gibbs free energy of the solute in aqueous solution, corrected just by the zero point energy [64]. The values obtained for the coefficient rates within this approach are reported in Table 3.

From data reported in Table 3, it can be seen that Model 2 gives the rate coefficients closest to the experimental values. The values found for k_α. and k_β are in very good agreement with the experiment when the *effective free energy* concept is adopted (disregarding the rotamer interconversion during the mutarotation), that corresponds to the Model 2 (GT +GG) values. Anyway, the search for a full numerical agreement can be misleading in this case, where the accuracy of the theoretical calculation (approximately 1 kcal/mol) is exponentially related to the rate coefficient. Besides, it is important to notice that only two glucose conformers were considered.

Table 3 Rate coefficients (k) for the glucose mutarotation, in seg^{-1}, assuming the reaction obeys a pseudo first order kinetics

	k_α	k_β
Model 1	3.6043×10^{-18}	8.5036×10^{-19}
Model 2 (GT)	3.6091×10^{-7}	1.7845×10^{-5}
Model 2 (GG)	6.6612×10^{-5}	7.6988×10^{-5}
Model 2 (GG+GT)	6.6473×10^{-5}	9.4833×10^{-5}
Exp.[a]	3.5167×10^{-4}	2.3389×10^{-4}
Exp.[b]	2.3233×10^{-4}	1.3667×10^{-4}
Exp.[c]	4.0546×10^{-4}	2.412×10^{-4}

The rate coefficients k_α and k_β stand for the α-pyr $\rightarrow$ α-TS barrier and β-pyr $\rightarrow$ β-TS barrier, respectively (see Fig. 9)
[a] Ref. [49]
[b] Ref. [44]
[c] Ref. [45]

Other structures are also present in solution, which were not considered in this work, contributing to the existence of other reaction pathways and consequently to the increase of k.

Another important question that is not the *focus* here, but deserves some attention is the number of the solvent molecules that has to be considered explicitly, besides the dielectric continuum, to obtain an acceptable agreement between theory and experiment. This information is an important piece of the physical model that must be described by the mathematical model, if one wants to match both experimental and theoretical results. However, from the data obtained and from the work of Yamabe and Ishikawa [56], it can be said that the first water molecule plays a decisive role in the process, changing completely the mutarotation mechanism, while the second and the third water molecules stand basically for the bulk effects (A.M. Silva et al. submitted)

4 Properties

4.1 General remarks

Historically, optical rotation (OR) has been the property associated with carbohydrates due to the high number of chiral centers present in such compounds. Compared to other spectroscopic quantities, the experimental determination of OR values is relatively straightforward, and this has contributed to the extensive study of all saccharides by OR during the last century.

Considering both, we report here the relevance of the OR property by itself, and in particular in the conformational study of carbohydrates to validate structures theoretically obtained, here the possibility of obtaining the OR values for glucose in aqueous solution using accurate ab initio methods. This investigation has several intrinsic difficulties to be overcome concerning different aspects: the sensitiveness of the OR theoretical values to the computational method used, the suitable description of the solvent effects and finally the high flexibility of the system studied, which makes glucose occur in many different possible conformations. A very detailed description of how each difficulty was overcome can be found elsewhere [65].

4.2 Methodology

Sampling the large conformational space of carbohydrates complicates the prediction of OR, especially when the energy differences of the most stable structures are of the same order as the accuracy of the theoretical models employed. Despite these difficulties, it has been determined that glucose occurs in aqueous solution with more than 99% as a six-member pyranosidic ring, in a stable $^4C_1$ chair conformation [66,67]. Several ab initio studies have been reported on the orientation of the hydroxymethyl and hydroxyl groups. Barrows et al. [52] have selected the structures used in their work from a set of 729 different starting geometries obtained

using the MM3(96) force field. They sampled the potential energy surface of glucose in the gas-phase, using a force field chemometrically tested [18]. This sampling resulted in a set of 13 final conformers, with the most stable ones being those that present the cooperative clockwise and anticlockwise orientation for the hydroxyl groups. Ma and collaborators found similar results [68]. Polavarapu and Ewig [69] showed that conformations with this cooperative effect disrupted have higher energies and are therefore much less abundant.

When we pass to aqueous solution, experiments seem to indicate that this concerted structure of intramolecular H-bonds is perturbed by the presence of donor and acceptor H-bonding waters [70,71]. More recently, Klein [72,73] showed that the hydroxyl groups can remain anticlockwise oriented in water even if intermolecular H-bond between some waters and the glucose hydroxyl groups are formed. Thus, the concerted clockwise and anticlockwise orientation of the hydroxyl groups does not necessarily exclude intermolecular hydrogen bonds with solvent, on the contrary it confirms the importance of cooperativity effects also in aqueous solution.

On the basis of these analyses we have defined our final set of eight conformers by combining the six structures (αGG, αGT, αTG, βGG, βGT, βTG) found by Allinger and coworkers [68,74] as the most stable conformers in gas-phase and the two additional conformations found to be also important in the quoted study of Barrows et al. [52] and here named βGG′ and βGT′.

The geometries of all conformers were obtained in gas-phase at B3LYP/6-31G(d, p)(5D) level. This same level was used to compute frequencies and thermal and entropic corrections to the free energy in the gas-phase, while the B3LYP/6-311G++(2d, 2p) level was used to obtain the energy ordering in the gas phase and in the aqueous solution.

4.3 Results and discussion

The structures of the eight conformers used in this work are shown in Fig. 10. The solvent effects were introduced through the IEFPCM [24,25] version of PCM [26,27]. The Boltzmann populations in the gas phase and aqueous solution were obtained for each conformer, after the introduction of thermal and entropic corrections.

Optical rotation (OR) values were calculated for the eight structures in water following the TD-DFT/GIAO approach described previously [65]. The results obtained with two different basis sets are reported in Table 4 as specific rotation $[\alpha]_D$ calculated at the sodium D line frequency (we recall that the specific rotation $[\alpha]_D$ is the observed rotation that is corrected for concentration (g/ml) and a defined path length (dm), namely $[\alpha]_D = \alpha_{obs}/(c \times l)$). The population of each conformer in the gas phase and in aqueous solution is also reported.

The results show that all α structures give a large positive contribution to the global $[\alpha]_D$, while the β structures give both positive and negative contributions. However, the most abundant β anomers (βGT′ and βGG) always give a negative contribution to the $[\alpha]_D$ net value. From data in Table 4, it is also interesting to note that the sign of the $[\alpha]_D$ is closely related to the anomeric hydroxyl position: both β anomers with the hydroxyl group upward oriented (βGG′ and βGT) present a positive $[\alpha]_D$ value. A detailed analysis of the importance of the various conformers and their different chiral centers on the $[\alpha]_D$ of glucose will be presented elsewhere (C.O. Silva, in preparation).

Considering the calculated Boltzmann weighted $[\alpha]_D$ value obtained, its agreement to the experiment can be considered quite good. We obtained 62.56 with the aug-cc-pVDZ basis set and 58.75 with the 6-31++G(d, p) basis set, which is fairly close to the experimental value of 52.7 [75]. We

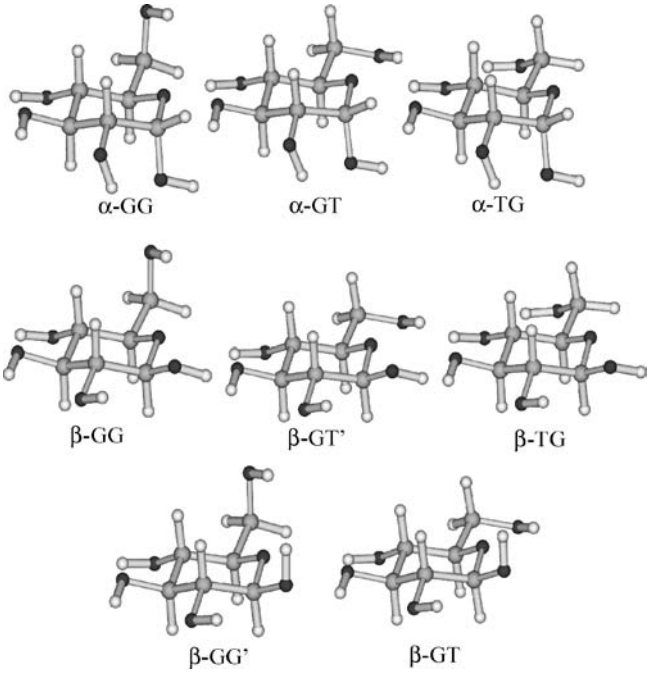

Fig. 10 Eight most stable conformers used to calculate OR of glucose in water

Table 4 Specific rotations $[\alpha]_D$ calculated in aqueous solution (in deg/(dm(g/cm^3)))

	aug-cc-pVDZ	6-31++G(d.p)	p_i^{gas} %	p_i^{sol} %
αGG	160.72	149.98	21	11
αGT	137.94	129.41	25	29
αTG	119.77	109.57	11	2
βGG	−10.16	−9.79	15	7
βGT′	−2.38	−4.03	13	37
βTG	−46.72	−46.76	7	3
βGG′	68.70	72.28	3	9
βGT	78.51	79.40	5	–
< >	62.56	58.75		
Exp.[a]	52.7			

The < > values stand for a Boltzmann weighted average in aqueous solution. The individual values for the Boltzmann population are reported in the gas phase and aqueous solution in the last two columns

[a] Ref. [75]

note that the weighted values using gas-phase populations are 82.31 and 76.82, respectively. The shift is the result of the α population dominating in gas-phase, while the β population dominates in solution. Since both anomers have very different $[\alpha]_D$ values (including the sign, with the the α anomers always large and positive), the inclusion of the solvent effects in the calculation of the relative energies of the various conformers is crucial. On the other hand, the solvation effect on the $[\alpha]_D$ of an individual conformer is less significant, and in fact always less than 5% .

Additionally, the validity of our approach is supported by the good agreement between the experimental and the theoretical OR values. From such an agreement, it can be inferred that the geometries of the most stable conformers of glucose seem to be determined by intramolecular stereoelectronic effects, which are already present in gas phase structures, and that are not overwhelmed by environmental effects like the solute–solvent interactions that take place in aqueous solution. For glucose these latter have an important influence on the energetic of the system changing basically the population distribution obtained in the gas-phase, but they are not able to generate new stable structures which are not already present in the gas-phase [52–57].

5 Concluding remarks

Three different situations where quantum chemistry was applied in the study of carbohydrate chemistry were reported in this work.

In the first one, conformational maps in gas phase and aqueous solution were presented for a disaccharide coming from two different approaches. The ab initio calculations furnished conformational maps and samplings in their most stable regions were performed. The selected structures were validated through the comparison of the $^3J_{H,C}$ property to its corresponding experimental value.

The possibility of replacing a relaxed map by a rigid one is important for two reasons: the time saved in the calculations and the qualitative evaluation of the intensity of the effects that determine the ϕ [76] and ψ [76,77] angles. Once these effects are strong enough, they are present also in the rigid approach for a conformational map, every time that the number of excessively hindered structures that has to be eliminated is not large enough to alter the topology of the potential energy surface studied.

In the second situation, the conversion between α and β pyranosidic forms of glucose in aqueous solution through an open aldehyde, known as mutarotation, was investigated. Two different mechanisms were studied, and through the comparison between the rate coefficients theoretically obtained for each mechanism with the experimental value, one pathway can be completely disregarded.

In the third situation, the optical rotation was calculated in aqueous solution at room temperature. It was possible to evaluate the individual contribution from each structure, and maybe in a near future it will be possible to use this information to get an idea of the hydroxyl orientation groups in a carbohydrate molecule.

The common aspect of these three situations is the fact that quantum mechanics offered structural or mechanistical information about the system, in pretty good agreement with an experimental property related to the process studied.

Nowadays, quantum mechanics does more than merely just describe model systems or small textbook-like molecules. The three examples reported show that it is applicable to real systems of chemical and biochemical interest as complex as carbohydrates. Moreover, it is a theoretical method able to describe electronic structure and the main electronic effects (as the anomeric effect) quantitatively better than any other theoretical approach, and thus properly describe systems in chemical situations where such effects play a decisive role.

Taking into account all the previous ideas, we sincerely believe that in a near future the quantum mechanics will help very much in the elucidation of many mechanisms where molecules as large as oligasaccharides are involved, bringing answers and contributing to the understanding of biological processes thanks to its unique ability to describe electronic structure.

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