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Ab initio conformational maps for disaccharides in gas phase and aqueous solution

Clarissa O. da Silva[a,*] and Marco A. C. Nascimento[b]

[a]*Departamento de Química da Universidade, Federal Rural do Rio de Janeiro, BR-465, Km 47, Seropédica, Rio de Janeiro CEP 23.890-000, Brazil*

[b]*Departamento de Físico-Química do Instituto, de Química da Universidade Federal di Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, RJ-CEP 24949-900, Brazil*

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Abstract—Ab initio conformational maps for β-lactose in both the gas phase and in aqueous solution have been constructed at the HF/6-31G(d,p) level of calculation. The results of the gas-phase ab initio calculations allows us to conclude that a rigid conformational map is able to predict the regions of the minima in the potential energy surface of β-lactose, in full agreement with those found in the relaxed conformational map. The solvation effects do not give rise to any new local minimum in the potential energy surface of β-lactose, but just change the relative Boltzmann populations of the conformers found in the gas-phase calculations. The values obtained for heteronuclear spin coupling constant ($^3J_{H,C}$), using the seven most stable conformers in solution are in good agreement with the available experimental values. This is a good indication that ab initio rigid conformational maps can be reliably used to sort the most stable conformers of β-lactose.

Keywords: β-Lactose; Conformational map; Disaccharide structure; Ab initio conformational map; β-Lactose conformers

1. Introduction

In this work we are interested in developing a fully ab initio procedure able to reliably select relative orientations of the monosaccharide units in the most stable conformers of a disaccharide[1,2] in aqueous solution.

Many important papers devoted to similar purposes have been published, but since it is not our intention to present a review on the subject, just a few of them—those closely related to the main goal of this paper—will be cited. The majority of the cited papers use classical methods, such as molecular mechanics, to investigate the structure of the conformers.[3–13] Many specific force fields have been developed in order to describe particular interactions present in saccharides.[14–20] More recently, a few papers using ab initio procedures to study the conformation of saccharides have started to appear in the literature.[21–24]

According to many authors, the most important geometrical parameters to define the three-dimensional structure of disaccharides are the dihedral angles of the glycosidic linkage[25,26] (Fig. 1), although the orientation of the secondary hydroxyl groups as well as the hydroxymethyl groups are also needed to completely define the whole structure of all possible conformers. The hydroxyl groups may be responsible for a large density of minima in the disaccharide potential energy surface; however we believe they are not as decisive as the angles ϕ and ψ in defining the more stable conformers. Therefore, we will be mainly interested in developing a fully ab initio strategy for finding such dihedral angles for the most stable conformers, assuming that the dihedral angles ϕ and ψ are the parameters that do define the mutual orientation of the monosaccharide units. The disaccharide chosen for this study is the β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranose, referred in this paper just as β-lactose (Fig. 1).

* Corresponding author. Tel./fax: +55-212-682-2807; e-mail: clarissa-dq@ufrrj.br

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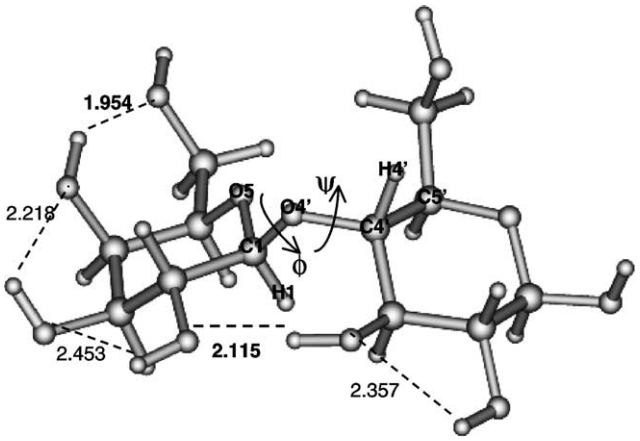

Figure 1. β-lactose in gas phase, in the *GGGG* conformation of the hydroxymethyl groups. The glycosidic linkage is defined by the values of the dihedral angles ϕ and ψ. The distances reported are in Å.

Our choice for ab initio procedures is justified by the nature of the interactions that define the dihedral angles of the glycosidic linkage. According to previous studies, it appears that, while the ψ angle is determined by nonbonded interactions, the ϕ angle is defined by the *exo*-anomeric effect.[26] Both interactions are very difficult to be properly taken into account by classical procedures, since they are very specific and difficult to be classically described, as can be inferred from the detailed work by Woods.[27,28]

On the other hand, ab initio techniques are not so simple to use when dealing with compounds that present a variety of conformers such as carbohydrates. Conformational maps are difficult to access at this level since many local minima may exist, which can masquerade each other, as well as the global minimum. Thus, this possibility must be taken into account when making use of ab initio procedures. Besides that, it is also important to remember that ab initio calculations are much more time demanding than classical procedures such as molecular mechanics. Therefore, it would be convenient to establish an ab initio protocol, as simple as possible, to be used for systems as particular as disaccharides.

In a similar context, an interesting comparison between relaxed and rigid conformational maps obtained from molecular mechanics was presented for cellobiose and maltose some years ago. French[6] has shown, among others things, that conformational maps generated by such distinct procedures furnish minima positions practically in the same regions. However, since molecular mechanics and ab initio calculations are completely different in their formulations, it is not possible to anticipate that the same behavior should result from ab initio calculations.

Therefore, we decided to investigate the similarities between ab initio relaxed conformational maps and those generated by the rigid-residue approximation, both calculated from first principles.

Another question that is generally present in conformational studies of carbohydrates is related to the solvent effects, mainly when the solvent is water. The interaction of any carbohydrate with water is expected to be mostly governed by electrostatic effects, due to the high dielectric constant of water and the high dipole moments of the hydroxymethyl groups present in a large number in the carbohydrate structure. Hydrogen-bond interactions, although with well-defined orientations can also be included among the electrostatic interactions since, as suggested,[29] its largest component is of electrostatic nature.

In order to investigate all the points mentioned above, we have constructed rigid and relaxed conformational maps for the β-lactose molecule in the gas phase, as well as in aqueous solution, adopting in this case a polarizable continuum description of the solvent. Finally, the validity of such a procedure to obtain the most stable conformers of disaccharides was tested by computing the heteronuclear spin coupling constants ($^3J_{C,H}$) across the glycosidic linkage, a property very sensitive to small variations of the angles (ϕ, ψ).

2. Methods

2.1. Calculations

Conformational maps were calculated for the β-lactose in gas phase at the HF/6-31G(d,p) level of calculation. According to Lii et al.[30] this basis set in a 5D form at the B3LYP level of calculation, gives reasonable good structures, but it overestimates stabilization by hydrogen bonds. They also suggested that using diffuse functions and larger basis (B3LYP/6-311++G**), most of the basis set superposition errors can be avoided. Before that, Csonka et al.[31] have established the importance of considering diffuse functions to avoid errors in calculating the energy when electronic correlation effects are taken into account. Since the main purpose of the present work is that of establishing the simplest possible ab initio protocol to generate reliable conformational maps for disaccharides, and since this will necessarily involve the construction of many conformational maps, we adopted the HF/6-31G(d,p) level of calculation. However, correlation effects have been also considered at the B3LYP level of calculation.

To introduce the solvent effects we used the Polarizable Continuum Model[32,33] in its Integral Equation Formalism (IEFPCM) formulation.[34,35] This solvation model has been successfully used in the study of many important physical–chemical properties,[36] and it has been proven to be a robust model to describe the solvation energy of many different systems.[37] In this approach, the solvent is described as a dielectric continuum medium, polarized by the presence of the

solute. A term representing the electrostatic interaction between the solute and the solvent is added to the solute Hamiltonian. The resulting equation, solved self-consistently, furnishes the electrostatic contribution to the solvation energy and the solute wave function modified by the solvent due to their mutual polarization. In this model the solute molecule is placed inside a cavity, opened in this dielectric, whose shape takes into account the geometry of the molecule. The polarization charges that mimic the solvent polarization due to the presence of the solute are located on the surface of such cavity. In the present case, the molecular cavities were built from interlocking spheres centered on selected atoms or group of atoms. The radii of the spheres are as follows: 2.28 Å for a CH or CH_2 group, 1.80 Å for an O atom, and 1.44 Å for an H bonded to oxygen atom of the hydroxyl groups.

All the gas-phase calculations were performed using the Jaguar program,[38] and for the calculations in solution we used the Gaussian98 code.[39] The conformational maps were generated from the interpolation of the respective energy data sets using the Radial Basis Function method.[22]

3. Starting structures and maps generation

The initial geometry of β-lactose used in this work was obtained as follows. β-D-glucose and β-D-galactose were built and then minimized using the Dreiding II force field.[40] These two structures were connected through a β-(1 → 4)-linkage, creating the desired compound. This structure was optimized in a fully relaxed calculation at the HF/6-31G(d,p) level, and the final geometry obtained is shown in Figure 1. In this optimized structure both hydroxymethyl groups present a GG orientation, while all the secondary hydroxyl groups are clockwise oriented. Some distances between atoms that could establish a hydrogen bond are reported. Those distances, whose values are within the ones normally found for hydrogen bonds, are in bold type. This structure presents dihedral angles of 256.07° (ϕ = O-5–C-1–O-4′–C-4′) and 63.96° (ψ = C-1–O-4′–C-4′–C-5′) and was used as the starting structure to built the conformational maps in all the calculations, unless otherwise specified.

Tvaroška and Pérez[41] have showed that the use of a starting geometry that corresponds to a local minimum can influence the shape of the potential energy surface of the compound by overstabilizing conformers in the region corresponding to that minimum. This effect could lead to wrong conclusions about the relative ordering of the conformers since this analysis is performed by selecting those structures in the conformational map that correspond to the minima. The most common procedure to avoid this artificial overstabilization is to adopt several different starting structures. However, a different procedure was used in this work, not just to avoid such overstabilization, but also to investigate the importance of the hydroxymethyl groups for the definition of the glycosidic angles, as will be made clear in the next sections. The procedure makes use of the same starting structure to generate three different conformational maps in the gas phase: one conformational map based on the rigid-residue approximation and two conformational maps based on a full relaxation of all geometric parameters of the carbohydrate.

The rigid conformational map was calculated for β-lactose starting from the fully relaxed geometry obtained at the same level. Rigid rotations were imposed to each glycosidic dihedral angle (ϕ and ψ) in increments of 30°, defined according to the sequence of atoms ϕ = O-5–C-1–O-4′–C-4′ and ψ = C-1–O-4′–C-4′–C-5′. From the 144 points obtained, 12 structures were eliminated from the set as being too hindered to be interpolated according to an energy criterion. The choice of an upper limit for the energy automatically eliminates those points of the map corresponding to conformations obtained from rigid rotations of both angles. Consequently, all the information pertained to that region of the conformational map becomes inaccessible. As a result the peaks in the regions of maximum energy were eliminated.

Relaxed conformational maps were also built for β-lactose by interpolating a set of data comprising 144 energy values, which was also generated by varying each dihedral angle in increments of 30°. For these two maps, the ϕ and ψ angles, once set to specific values, were kept frozen while optimizing all the other geometrical parameters. One of the relaxed maps was obtained using as the starting geometry for a given step the converged structure obtained in the previous one, while for the other map, as suggested by French,[7] the starting geometry for each step was created by rigid rotations of the glycosidic dihedral angles ϕ and ψ from the same initial structure reported in Figure 1.

In order to investigate the effects of electronic correlation on the conformational maps of β-lactose, a rigid map was calculated at B3LYP level, using the previously described procedure to construct the HF map, within the rigid-residue approximation.

The procedure used to calculate the rigid conformational map of β-lactose in the gas phase was repeated to construct a rigid conformational map in aqueous solution. The starting structure obtained in the gas phase was fully optimized in the solvent, considering just the electrostatic component of the solvation energy. This starting structure, shown in Figure 2, has dihedral angle values of ϕ = 252.03° and ψ = 63.52°, which define the relative orientation of the monosaccharide units. All the other points used to construct the conformational map in aqueous solution were also obtained considering just the electrostatic component of the solvation energy.

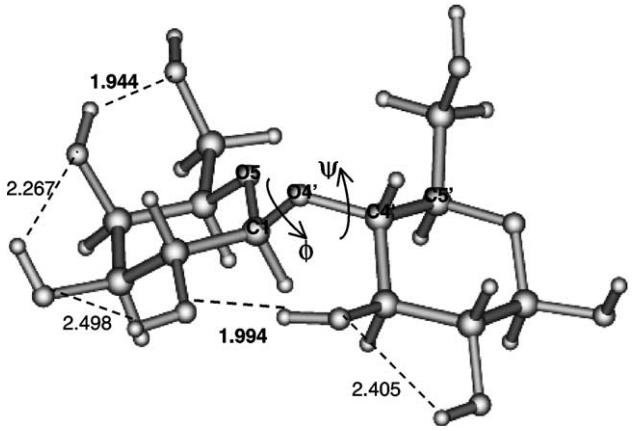

Figure 2. β-lactose in solution, in the *GGGG* conformation of the hydroxymethyl groups. The glycosidic linkage is defined by the values of the dihedral angles ϕ and ψ. The distances reported are in Å.

Comparing this geometry with that obtained for the correspondent structure in gas phase, we can say that no appreciable modifications were found. There are, however, some noticeable changes in the relative distances between groups that can interact through hydrogen bond. From Figures 1 and 2 it is clear that for those groups that, in the gas phase, interact more strongly (shorter O–H distances in bold face), the interaction was magnified in solution, while for the other groups the effect of the solvent was just the opposite.

Considering this new starting structure, rigid rotations were imposed in steps of 30° for each dihedral angle, exactly as in the gas phase. It is important to emphasize that each one of the 144 grid points obtained were calculated considering just the electrostatic solvent effects.

4. Results and discussion

4.1. Rigid × relaxed conformational maps in the gas phase

Rigid and relaxed maps, for β-lactose, were built according to the procedures described in the previous section.

The conformational map calculated for β-lactose within the rigid-residue approximation is shown in Figure 3. The gray-shaded regions of the potential energy surface are those made inaccessible by the elimination of the hindered structures.

From Figure 3, the points A, B and C correspond to regions of minimum and define the pair of values (ϕ and ψ) associated to the structures with the lowest energy values. On the other hand, from a previous study performed on the methoxyethoxymethane compound,[42] we know that the rigid-map approximation does not change the location of the minima in the conformational map,

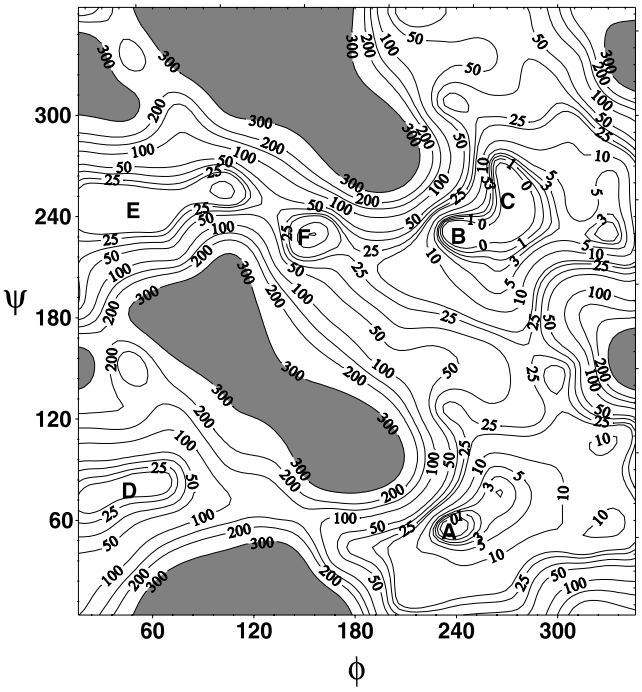

Figure 3. Rigid conformational map calculated for β-lactose at HF/6-31G(d,p) level (144 points). The energy values are in kcal/mol.

but just their relative energies. As a consequence, the rigid scanning would be able to properly locate the minima on the conformational map, but their relative energies will not be the same. Thus, positions D, E, and F cannot be excluded from the set of possible stable configurations.

Figures 4 and 5 show the relaxed maps obtained by rotations of the dihedral angles that define the glycosidic linkage, followed by the optimization of all the other geometrical parameters. Both relaxed approximations furnish energy minima practically in the same region. In fact, the maps are very similar, the main difference being related to the relative deepness of each region where the minima are located. Another difference between the maps of Figures 4 and 5 is in the region around $\phi = 150°$ and $\psi = 300°$, where Figure 5 shows contour lines of higher energy than in Figure 4.

To better compare the results obtained from the different approximations used to construct the conformational maps, we list, in Table 1, the approximate values of (ϕ, ψ) defining the six most stable regions for each map. It is important to mention that each region found as stable in the three conformational maps was rescanned in intervals of 10° for each dihedral angle.

Comparing the values reported in Table 1, it can be said that the rigid approximation is able to furnish regions of stability for the β-lactose that are also present in a relaxed conformational map, besides a few others not present in the relaxed maps. However, neither the depth of the local minima nor the height of the maxima ob-

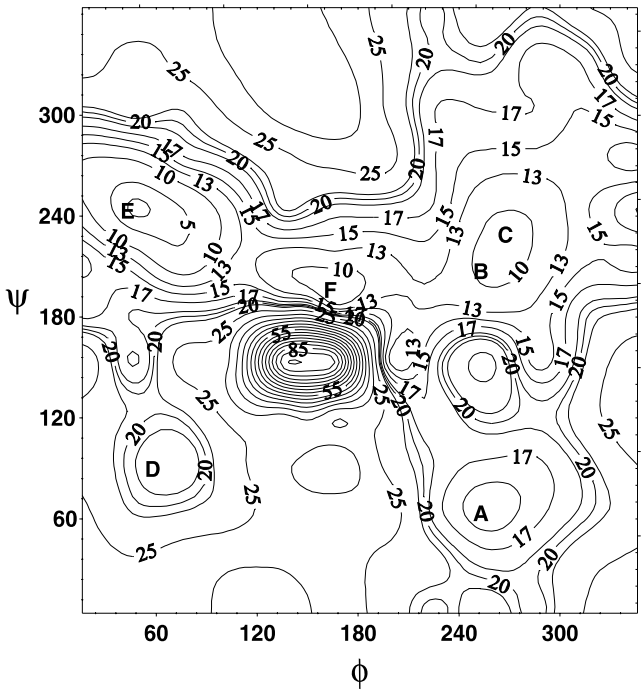

Figure 4. Relaxed conformational map calculated for β-lactose at HF/6-31G(d,p) (144 points) using in the successive steps, the geometry obtained in the previous one. Energies are in kcal/mol.

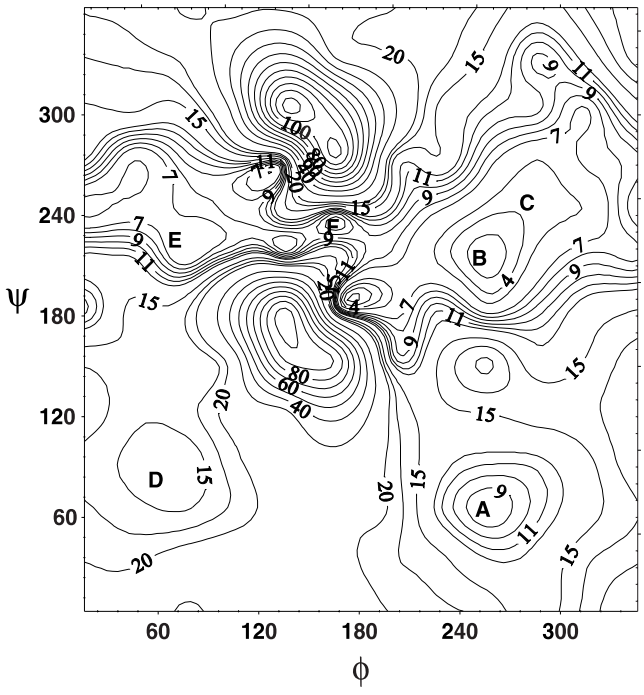

Figure 5. Relaxed conformational map calculated for β-lactose at HF/6-31G(d,p) (144 points), using as the starting geometry a structure constructed by rigid rotations of the ϕ and ψ angles of the geometry of Figure 1. Energies are in kcal/mol.

tained from the rigid description are comparable to those obtained from the relaxed one. Therefore, this approximation can be used, in principle, just to locate in the potential energy surface of this carbohydrate, pairs of dihedral angles that define the most stable mutual orientations of the monosaccharide units in a disaccharide.

Table 1. Position of the minima on the conformational maps calculated for β-lactose at HF/6-31G(d,p)

Conformer	Type of scanning					
	Rigid[a]		Relaxed[b]		Relaxed[c]	
	ϕ	Ψ	ϕ	Ψ	ϕ	Ψ
A	240°	55°	260°	60°	265°	70°
B	245°	230°	260°	220°	260°	235°
C	275°	245°	270°	240°	280°	245°
D	60°	80°	65°	90°	60°	90°
E	60°	245°	50°	250°	75°	240°
F	155°	230°	170°	200°	140°	220°

The values for the dihedral angles are in degrees.
[a] Minima of the map of Figure 3.
[b] Minima of the map of Figure 4.
[c] Minima of the map of Figure 5.

Figure 6 shows the conformational map for β-lactose considering electronic correlation effects through B3LYP calculations. The energy minima are almost all located at the same positions found when correlation effects were not taken into account (Fig. 3). The main difference observed was in the position of minimum C, which appeared on the map of Figure 6 in a region of higher ϕ value.

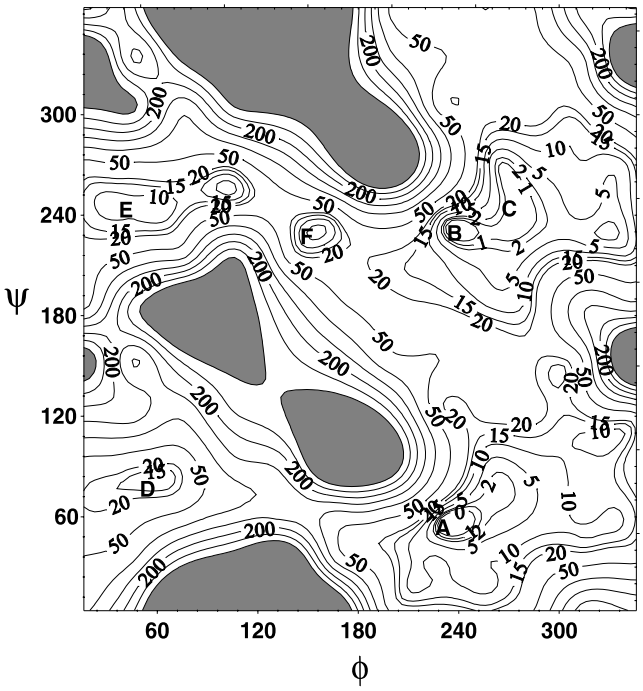

Figure 6. Rigid conformational map calculated for β-lactose at B3LYP/6-31G(d,p) level (144 points). The energy values are in kcal/mol.

4.2. Conformational map of the disaccharide in aqueous solution

The map for β-lactose in aqueous solution, constructed according to the procedure described in Section 2.1, is shown in Figure 7. Comparing maps of Figures 3 and 7, the regions of stability are approximately the same in gas phase and aqueous solution. For this carbohydrate, it seems that the interaction with the solvent will at most change the relative population of the conformers obtained in the gas phase, without generating new relative orientations of monosaccharide units, which were not already stable in the gas phase.

Since this is a rigid conformational map, obtained in exactly the same way as the one in the gas phase, some hindered structures were generated by rigid rotations of the glycosidic dihedral angles. Again the gray-shaded regions of the potential energy surface are those made inaccessible by the elimination of these structures.

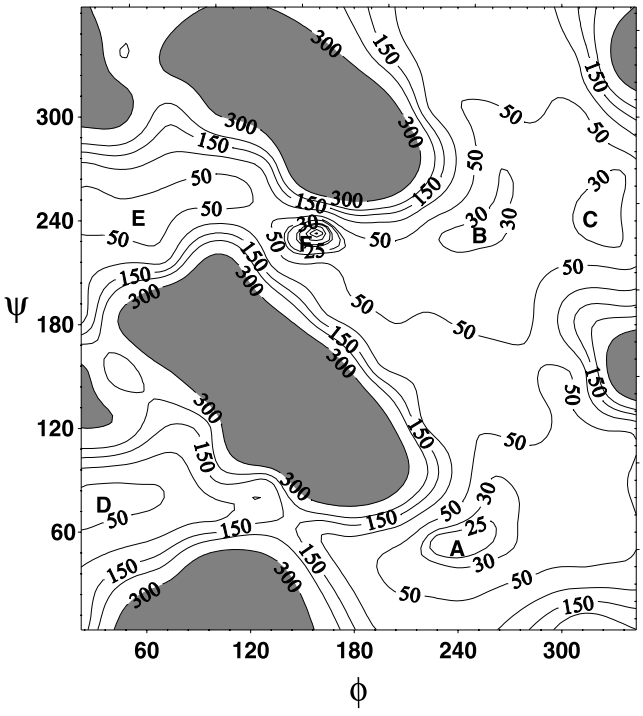

Figure 7. Rigid conformational map calculated for β-lactose at HF/6-31G(d,p) level in aqueous solution (144 points). Energies are in kcal/mol.

4.3. Validation of the rigid ab initio conformational map

In the previous sections, some regions of stability were found in a conformational map of β-lactose. These regions correspond to values of dihedral angles that define the most stable geometrical arrangements of the two rings that form the disaccharide. Once the more stable structures have been located on the potential energy surface of the β-lactose, it would be desirable to validate them. This can be done by calculating known properties of the compound, using these structures, and checking how well they can reproduce the respective experimental values. In order to do that, the six 'conformers' obtained from the rigid map were fully optimized at HF/6-31G(d,p) level, in two sequential steps. The 'conformers' were obtained imposing to the starting structure reported in Figure 1 the values of the dihedral angles shown in Table 1 for the rigid approximation. In the first step all geometrical parameters, except the dihedral angles ϕ and ψ, were relaxed. In the second step, these angles were also relaxed, and the geometry of the whole structure was optimized. During the energy optimization, conformer F was converted into conformer E. The values of the angles ϕ and ψ for the final five 'conformers' are shown in Table 2.

Also in Table 2, the relative internal energies (ΔU), the zero point energy corrections (ΔZPE) and the final free energy (ΔG) differences (after entropic and thermal corrections represented by $\Delta G^{(\text{rot–vib})}_{298\,\text{K}}$) are presented for the mentioned structures. The starting structure used in all conformational maps and located in the region A was not the most stable structure, contrarily to what would be expected.[41] From these data, just three of the five 'conformers' have significant Boltzmann populations in the gas phase at 298.15 K. These optimized structures are shown in Figure 8, with some few relevant geometrical parameters. Structures B and E exhibit basically two interresidue hydrogen bonds involving the same four atoms (labeling of atoms will follow the standard carbohydrate nomenclature): O-5···HO3′ and O-2···HO6′ in B and O-5···HO6′ and O-2···HO3′ in E.

Table 2. The ϕ and ψ dihedral angles (in degrees) and the relative energies (in kcal/mol) for five conformers obtained after full optimization of the geometrical parameters

Conformer	ϕ	ψ	ΔU	ΔZPE	$\Delta G^{(\text{rot–vib})}_{298\,\text{K}}$	ΔG^{b}	% Pop
A	256°	64°	+8.76	−1.80	−3.42	+3.54	—
B	263°	218°	+2.61	−1.23	−2.63	−1.25	25.80
C	283°	235°	+3.78	−1.89	−3.74	−1.85	71.07
D	71°	89°	+12.7	−1.49	−2.55	+8.68	—
E[a]	62°	242°	0.0	0.0	0.0	0.0	3.13

[a] The absolute energy values for conformer E in gas phase are: $U = -1290.720246$ a.u., ZPE = 254.58 kcal/mol, $\Delta G^{(\text{rot–vib})}_{298\,\text{K}} = 224.19$ kcal/mol.
[b] $\Delta G = \Delta U + \Delta\text{ZPE} + \Delta G^{(\text{rot–vib})}_{298\,\text{K}}$.

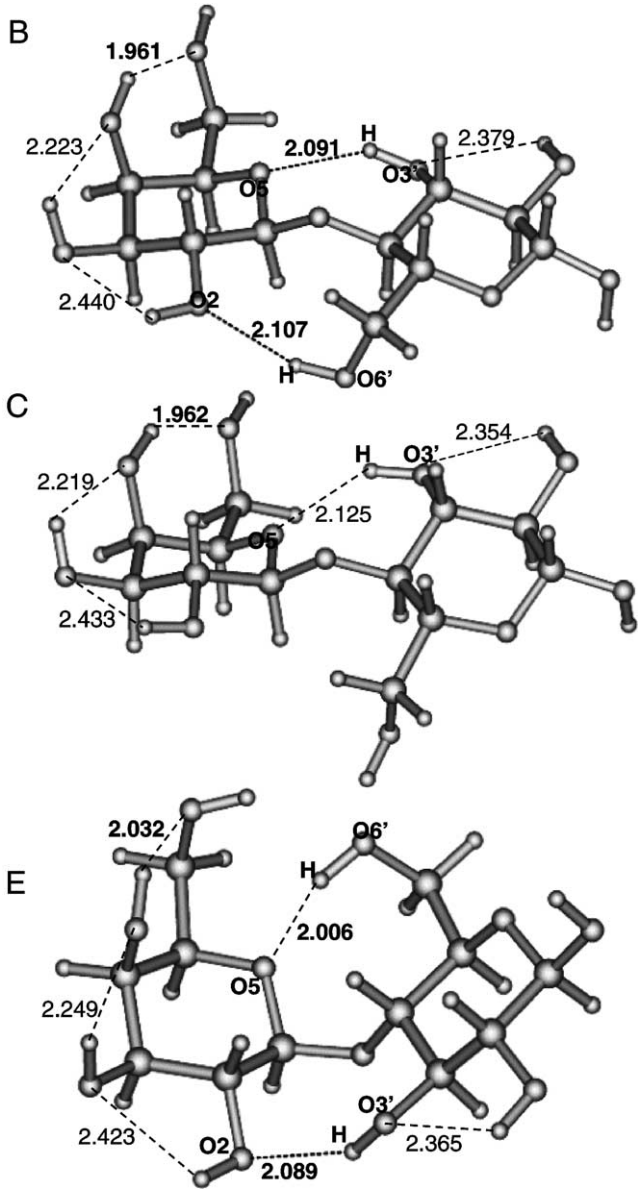

Figure 8. The three most abundant relative conformations of the monosaccharide units found in the gas phase. The labeling used is the same as that in Table 2. The distance values are in Å.

Conformer C exhibits just one interresidue hydrogen bond between O-5···HO3′ atoms, weaker than those present in the B and E structures. Besides that, the ϕ values found for conformers E and C are in reasonable agreement with those most stable values favored by the *exo*-anomeric effect.[43] The intraresidue hydrogen bond between the unique axially oriented hydroxyl and the hydroxymethyl group of galactose ring is present in all structures.

In order to consider solvent effects, the solvation energies were computed for the five conformers, using the respective geometries optimized in gas phase, at this time, considering also the additional nonelectrostatic[44] components of the solvation energy. These single-point calculations can be a reasonable approximation if one recalls that introducing solvent effects does not create any new stable region in the conformational map that was not already present in the map obtained in the gas phase (compare maps of Figs. 3 and 7) for this disaccharide. The final Boltzmann populations, after taking into account the solvation effects, are shown in Table 3.

Comparing the results in Tables 2 and 3, it can be seen that while conformer C is the most abundant in gas phase, followed by conformer B, in aqueous solution this pattern is reversed, conformer B being the most abundant structure in solution. However, since the difference in energy between these two conformers is smaller than the limits of accuracy of the methodology being used, one cannot in fact tell for sure, which one is the most stable structure. These results present a satisfactory agreement with the molecular dynamics[45] results obtained for the same system where the authors have defined the dihedral angles using a set of different atoms, involving the hydrogens of the glycosidic bonds. In their reference system, our dihedral angles that define the mutual orientation of the glucose and galactose moieties are, for the structure B, ϕ(H-1–C-1–O-1–C-4′) = 22.96° and ψ(H-4′–C-4′–O-1–C-1) = −23.96° and $\phi = 42.08°$ and $\psi = -5.67°$ for the structure C, while the values found by those authors are $\phi = 59.7°$ (±22.1°) and $\psi = -11.6°$ (±21.6°) for the structure in aqueous solution with the higher resident time.

In all calculations performed up to now, only the *GG* orientation of the hydroxymethyl groups of both monosaccharide units of glucose (Glc) and galactose (Gal) were considered. The most common orientations found for the hydroxymethyl groups in each moiety is *GG* and *GT*, the transition to *TG* being less observed due

Table 3. Components of the solvation energy (kcal/mol) for all conformers, and their respective corrections, obtained in gas phase

Conformer	ΔG_{electr}	$\Delta G_{nonelectr}$	ΔG_{solv}[a]	$\Delta G_{solv,298\,K}$[b]	% Pop
A	+3.63	+0.98	+4.61	−0.61	0.24
B	−0.37	+0.29	−0.08	−3.94	60.24
C	+0.70	+1.24	+1.94	−3.69	39.46
D	+8.19	+1.71	+9.90	+5.86	—
E[c]	0.0	0.0	0.0	0.0	0.06

[a] $\Delta G_{solv} = \Delta G_{electr} + \Delta G_{nonelectr}$.
[b] $\Delta G_{solv,298\,K} = \Delta G_{solv} + \Delta ZPE + \Delta G_{298\,K}^{(rot-vib)}$, the latter two terms are the same as that obtained in gas phase.
[c] The absolute solvation energy values for conformer E are $G_{electr} = -1290.755157$ a.u. and G_{total}(electr + nonelectr terms) = −1290.747164 a.u.

Table 4. Data involved in the calculation of the Boltzmann population in aqueous solution, at 298.15 K

	*GG*Gal*GG*Glc		*GT*Gal*GG*Glc		*GG*Gal*GT*Glc		*GT*Gal*GT*Glc	
	B[a]	C	B	C	B	C	B	C
ϕ	263	283	260	260	281	280	267	266
ψ	218	235	205	205	228	227	203	202
ΔU	0	+1.17	+2.49	+2.49	−0.20	+3.86	+2.37	+6.43
ΔZPE	0	−0.65	−0.41	−0.41	−0.42	−0.89	−0.72	−1.18
$\Delta G_{298\,K}^{(rot-vib)}$	0	−1.12	−0.41	−0.41	−0.85	−1.56	−1.12	−1.81
ΔG_{electr}	0	+1.08	−0.03	−0.04	+0.08	+1.72	+0.18	+1.94
$\Delta G_{nonelectr}$	0	+0.95	+0.64	+0.63	+0.60	+0.64	+0.83	+0.87
ΔG_{solv}[b]	0	+2.03	+0.61	+0.59	+0.68	+2.36	+1.01	+2.81
$\Delta G_{solv,298\,K}$[c]	0	+0.26	−0.21	−0.23	−0.59	−0.09	−0.83	−0.18
% Pop	8	5	11.89		21.84	9.37	32.75	10.91

Dihedral angles are in degrees, and energy values are in kcal/mol.

[a] The absolute energy values for conformer E in gas phase are: $U = -1290.716084$ a.u., ZPE = 253.35 kcal/mol, $\Delta G_{298\,K}^{(rot-vib)} = 221.56$ kcal/mol. In aqueous solution are $G_{elect} = -1290.755753$ a.u. and G_{total}(electr + nonelectr terms) $= -1290.747289$ a.u.

[b] $\Delta G_{solv} = \Delta G_{electr} + \Delta G_{nonelectr}$.

[c] $\Delta G_{solv,298\,K} = \Delta G_{solv} + \Delta ZPE + \Delta G_{298\,K}^{(rot-vib)}$, the latter two terms are the same as that obtained in the gas phase.

to a higher energy barrier.[8,45] In order to investigate the other possible orientations for the hydroxymethyl groups, and how they affect the dihedral angles of the glycosidic linkage, six new additional structures were investigated, assuming that the dihedral angles of structures A, D, and E are not favored at all. They were created from the optimized conformers B and C (the most abundant species in the gas phase and solution), which have both *GG*Gal*GG*Glc orientations. By proper rigid rotations, three new starting structures were created, named *GG*Gal*GT*Glc, *GT*Gal*GG*Glc, and *GT*Gal*GT*Glc for each conformer. These six new structures had their geometrical parameters optimized in the gas phase (HF/6-31G(d,p)), their analytical frequencies calculated, and also their respective single point solvation energy calculated. All the structures obtained kept their OH groups oriented clockwise. The energy values for the final structures obtained are shown in Table 4, together with the respective Boltzmann population in aqueous solution.

From Table 4, it can be seen that the six new additional conformers considered have a non-negligible population in aqueous solution. Structures B and C both converged, after optimization procedures to the same *GT*Gal*GG*Glc structure. The most abundant conformations for the hydroxymethyl groups seem to assume *GT*Gal*GT*Glc and *GG*Gal*GT*Glc orientations.

Experimental results[8] show a probability distribution for the different hydroxymethyl conformations of the glucose moiety of 60% for *GG* and 40% for *GT*, while for the galactose fragment the values are 70% for *GT* and 30% for *TG*, this last one not considered in this work.

From the two sets of energy values reported in Tables 3 and 4, some effects of group rotation on the total energy of the disaccharide can be sketched. In Table 3, the relative energies have an absolute mean value of 3.5 kcal/mol, while in Table 4 it is 0.4 kcal/mol. The former number refers to ring rotations around the glycosidic bond, and the latter to hydroxymethyl rotations around the C-5–C-6 (or C-5′–C-6′) bonds. Comparing such numbers, it can be said that the first type of rotation has a more drastic effect over the whole energy of the disaccharide than the last one.

In order to validate the conformational map for β-lactose within the rigid scanning procedure, the heteronuclear spin coupling constant ($^3J_{H,C}$) was calculated across the glycosidic linkage using a Karplus-type equation for the C–O–C–H segment in the form:[38]

$$^3J_{H,C} = 5.7\cos^2(\Phi^H) - 0.6\cos(\Phi^H) + 0.5,$$

where

$$\Phi^H = (\text{H-1–C-1–O-1–C-4}').$$

This property was chosen due to the fact that it is very sensitive to small variations of the angles (ϕ, ψ) and also because it just depends on the glycosidic angles (ϕ, ψ) that define the conformation of the β-lactose. The values obtained for each conformer are reported in Table 5. The atom labeling follows that of Figure 1.

In order to establish a comparison with the known experimental values for this property, the respective Boltmann populations obtained in aqueous solution, for each conformer, were used in a weighted average calculation of $^3J_{H,C}$, and the results are shown in Table 6, together with the experimental values. Also reported in Table 6 are other values for $^3J_{H,C}$ computed from different sets of conformers, obtained from classical conformational maps.

The data reported in Table 6 shows that the approximation tested in this work was able to generate values of $^3J_{H,C}$ very close to the experimental values, and better than those obtained from classical methods. The number of the conformers is also impressively smaller than

Table 5. $^3J_{H,C}$ values for the conformers of β-lactose studied in this work and their respective dihedral angles, in degrees

	*GG*Gal*GG*Glc		*GT*Gal*GG*Glc	*GG*Gal*GT*Glc		*GT*Gal*GT*Glc	
	B	C	B or C[a]	B	C	B	C
Φ_1 = (H-1–C-1–O-1–C-4′)	22.96	42.08	20.23	40.96	39.36	27.51	26.50
Φ_2 = (H-4′–C-4′–O-1–C-1)	−23.96	−5.67	−37.74	−11.57	−12.85	−38.39	−39.24
$^3J_{H,C}(\Phi_1)$	4.78	3.19	4.96	3.30	3.44	4.45	4.53
$^3J_{H,C}(\Phi_2)$	4.71	5.55	3.59	5.38	5.33	3.53	3.45

[a] In this case the B and C 'conformers' converge to the same final structure after full optimization.

Table 6. Final $^3J_{H,C}$ values for β-lactose,[10] methyl β-lactoside[39], and ethyl β-lactoside[7]

	This work	Exp[a]	Exp[b]	MM3[c]	PEF95[d]	PEF95G[e]
H-1,C-4′	4.13	3.8	3.7	3.5	3.8	3.6
H-4′,C-1	4.30	4.9	—	4.2	4.1	3.4

[a] Ref. 39.
[b] Ref. 7.
[c] Obtained from a Boltzmann distribution at 300 K from ΔE, using the complete MM3(92)[40,41] theoretical ensemble comprising 34,443 conformations (Ref. 7).

the number used to calculate the same property, when classical approaches are adopted.

It would be interesting to test the validity of the approach used to construct the conformational maps by calculating other properties of the molecule. However, this might not be a simple task inasmuch as most of the other properties will depend on the positions of the hydroxyl groups, whose most stable orientations were not investigated in the adopted approach. Nevertheless, the present results are very encouraging, and the construction of conformational maps for other disaccharides is in progress.

5. Conclusions

Ab initio conformational maps for β-lactose have been constructed at HF/6-31G(d,p) and B3LYP/6-31G(d,p) calculation levels in the gas phase and at HF/6-31G(d,p) calculation level in solution, using different procedures.

The results of the gas phase ab initio calculations allow us to conclude that a rigid conformational map is able to predict the regions of the minima in the potential energy surface of β-lactose in full agreement with those found in the relaxed conformational map.

The map obtained in aqueous solution shows regions of minimum energy in the same positions of the regions found in the map calculated in the gas phase. It may be an indication that the solvent effects, as described by the PCM approach, do not give rise to any new local minimum in the potential energy surface of β-lactose, but just change the relative Boltzmann populations of the conformers found in the gas phase calculations. Such findings were confirmed by relaxed conformational maps obtained in the gas phase and aqueous solution for a prototype molecule.[42]

The results obtained for heteronuclear spin coupling constant ($^3J_{H,C}$) provided a good test for the validity of the approach adopted. Therefore, based on the results obtained for the β-lactose molecule, one may conclude that rigid ab initio conformational maps can be successfully used to sort the most stable conformers of a disaccharide in both the gas and solution phases, once one assumes that the most important geometrical parameters defining the preferred conformations of the disaccharides are the dihedral angles of the glycosidic linkage.

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