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Efficient modelling protocols for oligosaccharides: from vacuum to solvent

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The determination of conformational preferences of oligosaccharides is best approached by describing their preferred conformations on potential energy surfaces as a function of the glycosidic linkage Φ, Ψ torsional angles. For proper molecular mechanics modelling the flexibility of the rotatable pendant groups must also be considered. The so called adiabatic maps partially mimic the flexibility within the 10 dimensional conformational space of the pendant groups of the given disaccharide. These molecular mechanics maps are considered to be the state-of-the art of the Φ, Ψ potential energy surface of disaccharides recently calculated. The RAMM (*RA*ndom *M*olecular *M*echanics) method was shown to be able to calculate such profiles automatically. Additionally, based on the continuum solvent approach, RAMM allows the calculation of the effects of solvent on conformational energy profiles. Molecular dynamics simulations are also useful tools to study the influence of solvent on conformational behaviour of oligosaccharides. The capability of the RAMM calculational protocol to locate low-energy conformers on the multidimensional potential energy hypersurfaces of disaccharides is illustrated and compared with molecular dynamics simulations with and without inclusion of the solvent.

***Keywords*: oligosaccharide conformation, modelling protocols, random molecular mechanics, molecular dynamics, solvent effect**

1. Introduction

Oligosaccharides have been found to have many biological functions, *eg* cell–cell recognition and related interactions [1] and the attachment of viruses to host cells [2]. Oligosaccharides also serve as ligands for many proteins. Steric and spatial considerations based on the shape (conformation) of the oligosaccharide molecules play a key role in understanding the principles of protein–sugar interactions. To get insight into rules of these interactions it is important to analyze which parts of the conformational space of carbohydrates are accessible. It has been proved that oligosaccharides generally exhibit several conformations [3–6] in equilibrium in solution. Efficient computational strategies are thus required to investigate the environmental effects on conformational equilibria of oligosaccharides in solution or for modelling their interactions with proteins.

The determination of conformational preferences of oligosaccharides is best approached by describing their prefered conformations on potential energy surfaces as a function of the glycosidic linkage Φ, Ψ torsional angles. In the early days of conformational analysis of oligosaccharides the calculations of either rigid or, later, relaxed conformational maps predominated. Today, complex simulation techniques such as molecular dynamics and random sampling are increasingly being used. Several examples can be found in the literature of 'state-of-the-art' conformational search strategies in order to simplify the complexity of the multiple-minima problem. Several relaxed maps were calculated for the construction of the 'adiabatic' potential energy surface (up to 14 for α-lactose [7]), from which the lowest-energy structure for each Φ_i, Ψ_j combination was selected. In the case of lactose, all of the secondary hydroxyl OH bonds were oriented to point in a clockwise or anticlockwise pattern (as viewed from above the pyranose ring), and four possible combinations of GG, GT (glucose) and TG, GT (galactose) hydroxymethyl rotamers were considered. The methodology presented recently by French and Dowd [8] (48 relaxed maps for sucrose) is clearly one of the most comprehensive systematic grid searches performed so

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far, but even with the computational power available today a similar protocol may be impractical for larger oligomers.

Molecular dynamics (MD) simulations are being increasingly applied to obtain the populations of all of the accessible (*ie* low-energy) conformations of oligosaccharides. Currently, MD simulations of oligosaccharides without solvent molecules as 'state-of-the-art' technique are reaching nanoseconds on the time scale. MD simulations provide an efficient tool for calculating the possible influence of solvent molecules on the conformational fluctuations of oligosaccharides. The conclusions, however, are conflicting. There are suggestions that motions in water have similar amplitudes as in vacuum simulations [9], are damped as in proteins [10] or even exhibit more flexibility within one local minimum [11]. Water is not the only solvent of interest for MD studies; other solvents are of interest for lipophilic structures for example, or may be required in NMR experiments for technical reasons or for solubility. Thus, the influence of these solvents on conformational behaviour must be studied. Mierke and Kessler [12] have parametrized various commonly used organic solvents on the basis of a united-atom approach and have successfully simulated the conformational behaviour of peptides in such solutions. Two simplifications make such studies feasible: (1) because of the lower volume density of organic solvents vs. water, fewer solvent molecules are needed to fill a given volume; (2) the united-atom approach, which regards a methyl group as one atom for example, reduces the number of atoms to be simulated (*eg* DMSO is reduced from 10 to 4 atoms). Thus MD simulations with organic solvents can be performed in much less time than the corresponding water simulation. MD runs of nanosecond duration are feasible with current desktop workstations.

In this paper we describe and discuss the advantages and limitations of those modelling techniques and protocols we found to be effective for exploring the conformational space of carbohydrates and which we have applied intensively to various oligosaccharides. A more comprehensive survey of other frequently used modelling protocols can be found elsewhere [13].

2. Computational methods

2.1. Random molecular mechanics (RAMM)

The RAMM (RAndom Molecular Mechanics) method [14] was proposed for the semiautomatic calculation of the n-dimensional potential energy surface (PES) of oligosaccharides and other biomolecules. The basic philosophy is to optimally combine three independent procedures: a grid-based conformational search for one pair of bonds, random generation of a conformational ensemble of all rotatable bonds and optimization of molecular geometry.

When dealing with oligosaccharides, a conformational ensemble is generated for each Φ_i, Ψ_j regular-grid point in a random manner, modelling the rotability of the pendant groups. An example of such modelling, with marked pendant groups, is given for maltose in Figure 1. From the conformational ensemble of rotatable pendant groups the lowest-energy conformation for each Φ_i, Ψ_j grid is selected for further processing. Due to the size of the generated ensemble *n* (optional parameter which can be set to several thousands) no geometry optimization has been included during the ensemble generation. The neglect of the geometry optimization has been shown to be a shortcoming of the method, because the potential energy surface of the molecule, calculated in non-optimized geometry differs from the surface following from optimized geometry. The method has been improved with partial geometry optimization of *l* low-energy conformations as the subset of *n*. The lowest-energy form from the pre-optimized *l* geometries was selected for the global geometry optimization of each grid point on the two-dimensional Φ, Ψ potential energy surface.

It is believed that this sampling procedure will find all possible local and global minima since rotational energy barriers can be crossed. Through the subsequent force field-based optimization of the geometry, it is likely that the energetically most favourable conformation for each forced Φ, Ψ combination is reached. In most cases this will be the nearest local minimum. Another advantage of this approach

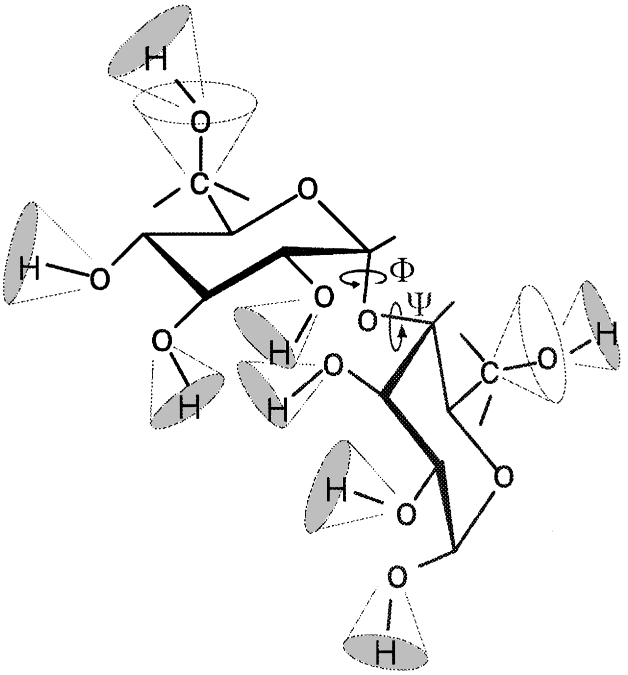

Figure 1. Strategy of the RAMM conformational analysis of maltose. Regular-grid search is performed around the glycosidic bond for the Φ, Ψ torsional angles with simultaneous generation of conformational ensembles modelling the mobility of the pendant groups. The last (schematically illustrated with cones) is achieved with a random-walk technique within the torsional angle space of the pendant groups.

is that neither explicit knowledge from experimental structural data nor from former computer experiments is necessary for the exploration of the conformational space of an oligosaccharide.

This procedure resembles the methodology of the construction of adiabatic maps, but has several advantages: the complete calculation process is automatic and all rotatable bonds in the molecule (*eg* 10 bonds in an ordinary disaccharide in addition to Φ, Ψ) can be handled simultaneously. The method is not restricted to a conformational search for di- or trisaccharides. Two dimensional Φ, Ψ sub-profiles for larger oligosaccharides can be obtained from the total number of the $18 \times 18 \times n$ conformations generated for each disaccharide subunit. Another possibility of consequent conformational sampling is to generate n additional conformations for the given low-energy form of the oligosaccharide.

The force field used in the RAMM method follows the MM2 [15, 16] parametrization and force field.

2.2. Conformational clustering

The two-dimensional grid-constrained Φ, Ψ conformational profiles reasonably approximate the allowed regions in which the given torsional angles should fluctuate, when calculated as described above, reflecting the mobility of the pendant groups. Rasmussen and Fabricius [17] asked the question: '… what is the significance of these plots? – and what is their use?' and answered it in following way: '… a more rational use of computer facilities would probably be to chart valleys of the conformational map.' The difficulty associated with such approach is that in the case of more complex oligosaccharides it is usually difficult to forecast in advance the precise localization and shape of the valleys.

We propose here, as an extension of the RAMM protocol, to chart the entire two-dimensional conformational energy surface of the oligosaccharides by allowing the molecule to relax down-hill from each Φ and Ψ grid-constrained point to the nearest local energy minimum on the surface. The orientation of the pendant groups has already been attained for any *i*-th or *j*-th grid-constrained geometry of the Φ, Ψ surface, following the RAMM calculation. These structures are used as input to start the final, completely unconstrained optimization. This is a prerequisite for consequent clustering. The clustering is based on *rms* values of torsional angles of individual conformations. The members of the given cluster might have different or identical orientations of the pendant groups. Identical members of each cluster have to be eliminated in order to properly calculate the Boltzmann conformational equilibrium. The clusters of conformations such obtained should provide the most comprehensive estimation or the energetically allowed conformational space for the given glycosidic bond. In principle it is also possible to use the same methodology as outlined above to optimize all geometries generated during the molecular dynamics simulation of the given oligosaccharide and cluster the minimized geometries into conformational families.

2.3. Molecular dynamics (MD)

Molecular dynamics simulations involve the calculation of the dynamic motions of a system of atoms using the differential equations of motion in the chosen force field and series of 'infinitesimal' time increments. The time evolution of the molecular motions, so-called trajectories, can be used to study time-dependent properties, such as the population of various accessible conformers. Because kinetic energy is added to the complete molecular system by means of a temperature parameter, energy barriers on the order of kT can be crossed for each torsional degree of freedom. Thus, MD simulations allow molecules to traverse energy maxima and to populate different stable conformations. MD trajectories can be used to compare calculated population distributions with experimental conformation parameters derived from NMR measurements [18].

When explicit solvent molecules are included, the number of atoms increases from at most a few hundred to several thousand, with a corresponding increase in the number of equations of motions that must be evaluated. Since computational time increases with the square of the number of atoms involved, it is clear that for large molecules, solvated species or complexes, current computational resources limit the simulations to at most a few hundred ps, a time that may be much shorter than that required for the conformational equilibria that influence experimentally determined parameters.

For MD studies of oligosaccharides in solution the use of a periodic boundary conditions approach is required, *ie* a simulation of the molecule + solvent system as a periodic lattice of identical subunits. Periodic boundary conditions result in a much better representation of the bulk solvent, even for the interior of the cube. Solvent molecules do not diffuse away, and those molecules near the surfaces of the box interact with solvent molecules across the boundaries to adjacent cubes. Solvent molecules in the surrounding cubes are created by symmetry operations performed on the simulated atoms near the boundaries of the central cube. The mirrored or image molecules provide forces which restrain the solvent within the box and improve continuity at the boundaries so that a bulk solvent (without boundary effects) is simulated to a good approximation. Through the use of symmetry, trajectories need to be calculated only for molecules within the central box, and the calculation time is not significantly increased. Nevertheless, such simulations still require days to weeks of computation on high-performance computers. The reported MD simulations of oligosaccharides in water have been performed over time scales of a few tens of picoseconds [11, 19–22] and up to several hundred picoseconds [9, 10, 23, 24].

2.4. Continuum-solvent approach

In addition to molecular dynamics models of the solute–solvent interactions the more simple continuum solvent approach – a fast method, unpretentious for computer time consumptions – has been used to examine the influence of solvent on two-dimensional conformational energy profiles of the disaccharides under study. In this method, the total energy of the molecule comes from the energy of the solute ΔG_{vac} and solvation contribution ΔG_{solv} [25]:

$$\Delta G = \Delta G_{vac} + \Delta G_{solv} \tag{1}$$

The solvation energy itself is composed from cavitation, dispersion, electrostatic contributions, and a specific solute–solvent interaction term:

$$\Delta G_{solv} = \Delta G_{cav} + \Delta G_{disp} + \Delta G_{elst} + \Delta G_{spec} \tag{2}$$

The partial atomic charges of sugars, necessary for the solute–solvent to electrostatic interaction term, were obtained using the method of Mortier *et al.* [26]. Following the method of Mortier, the atomic charges are calculated on the basis of the principle of electronegativity equalization, a formalism that has been developed for the calculation of atomic charges in molecules which are connectivity and geometry dependent.

3. Results and discussion

The efficiency of the methods for prediction of regions occupied by stable conformations is illustrated on two, alpha glycosidically linked disaccharides: on the molecule of β-maltose and on molecule α-L-Fuc(1-2)β-D-Glc-1OMe.

The adiabatic potential energy surface of β-maltose, calculated with RAMM, is illustrated in Figure 2. No influence of solvent on the potential energy surface has been considered at this stage. The shape of the profile is similar to other maltose profiles, calculated with various force fields, known from literature [8, 27–29]. The main (global) minimum of the calculated surface is located around — 30/—30° and is stabilized via intramolecular O2 ... O3′ hydrogen

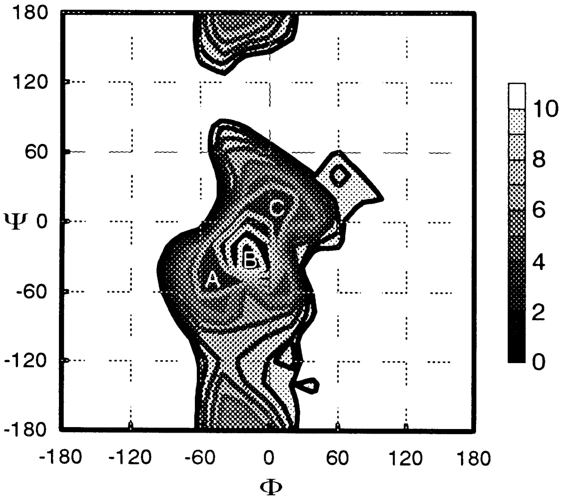

Figure 2. Relaxed potential energy map of maltose, calculated with RAMM. Dielectric constant 4 has been used for calculation of the electrostatic energy contribution.

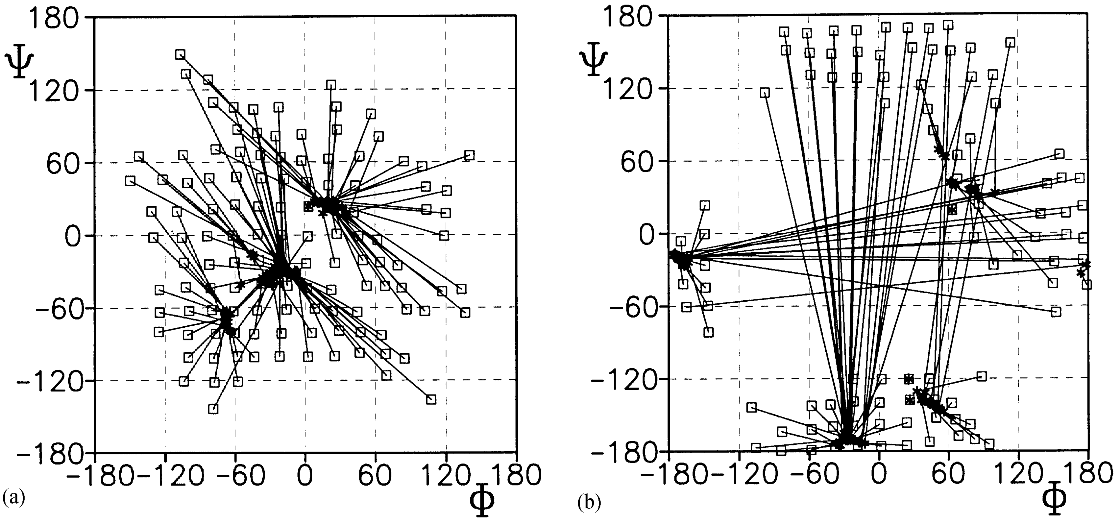

Figure 3. Conformational clustering of maltose. All glycosidic torsional angles of the Φ, Ψ surface were allowed to relax to the nearest energy minimum. The lines define the path between starting (squares) and optimized (asterisks) values. Only the conformationally allowed space defined by a threshold of 12 kcal mol^{-1} above the global energy minimum is shown. Seven different local minima were found by this approach. To improve the clearness of the presentation bunches of clusters have been grouped together and are displayed separately as (a) and (b). The dominant cluster (Φ, Ψ: – 20/ – 30) is represented (left).

bonding. This minimum corresponds to the minimum signed B in the related article by Tran *et al.* [28] on maltose. (The conformer terminology of Tran will be used consistently throughout this paper). Minima A and C are not clearly seen as local minima in Figure 2, but the regions, where they should be located, is limited to the 4 kcal mol^{-1} energy contour. There is an indication for additional minima on the surface, *eg* at 60/40° or at 40/—140°. The *trans* or *anti* conformation, located approximately at —20/—180° exhibits approximately 5 kcal mol^{-1} higher energy than is the energy of the global energy minimum.

The direct localization of all of the maltose local minima on the Φ, Ψ surface is evident from the clustering protocol and is illustrated in Figure 3. The straight lines between squares (belonging to a certain grid on the surface) and the asterisks indicate how the unconstrained optimization locates minima on the Φ, Ψ surface. The squares correspond to the starting geometry and the asterisks to the minima on the surface, resulting from optimization. In this way all minima discussed in the literature were found, including the minimum at 40/—140°, which was shown as a feature of CHARMM [27] but missing from the MM2-CARB

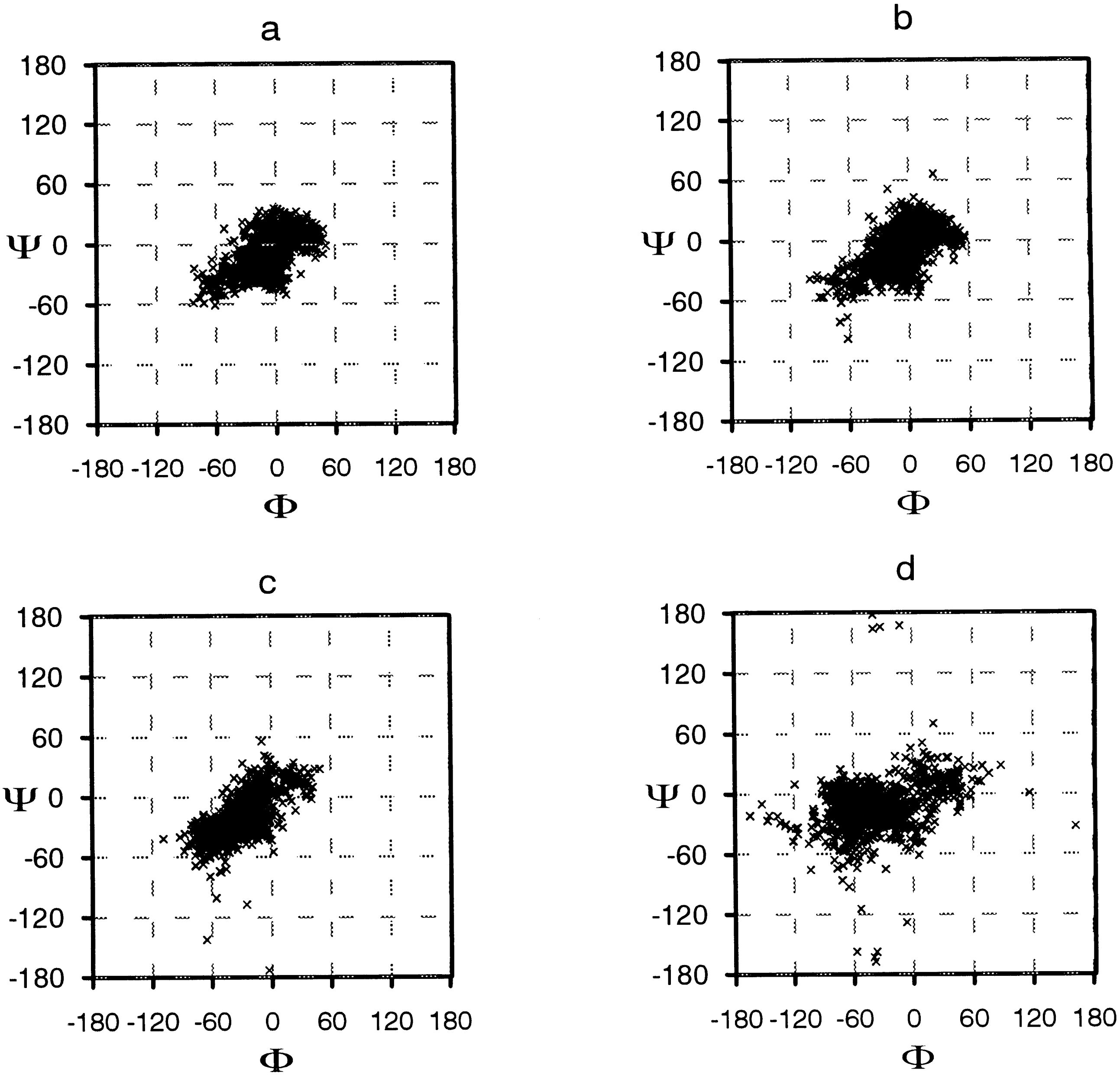

Figure 4. Molecular dynamics simulation (1.05 ns) of maltose calculated with the Discover program and CVFF force field. The influence of simulation temperature (300 K upper row, 400 K lower row) and dielectric constant used for electrostatic energy contribution ($\varepsilon = 1$ on a, c and $\varepsilon = 4$ on b, d on the Φ, Ψ profiles is shown.

conformational search [28]. We would like to emphasize that the difference between the latter study and our results is probably not due to the use of different force fields (MM2-CARB and MM2(87)) but can be attributed to variations in the applied conformational search protocol.

The global minimum from the adiabatic map (Figure 2) has not been shifted more than 10° during the four calculational sets we provided (both maltose anomers, alpha and beta and calculations with dielectric constant 1.5 and 4). The neighbouring regions of clusters of conformations, located around −60/−60° and 30/30° are now unambiguously present on the surface. Energy lowering has also been obtained for the *trans* conformation (located around −30/−170°). The relatively large flexibility of maltose can immediately been concluded from the presented figure, in which only those local energy-minimum clusters are shown whose relative energy is within the 12 kcal mol^{-1} limit above the global energy minimum of maltose.

The flexibility, at least in the A-B-C region, has also been confirmed by a 1 ns molecular dynamics simulation (Figure 4). The force field used in the MD-simulation, CVFF, differs significantly from the MM2(87), but the results of both compared methods are similar in view of the frequent conformational exchange within the A-B-C region. The flexibility resulting in the frame of the given region is independent of the simulation temperature or the value of the dielectric constant which has been used to calculate the electrostatic contribution. The only questionable simulation from the given example is the one at 400 K and $\varepsilon = 4$ (Figure 4d) where $^4C_1$–$^1C_4$ ring interconversions have been observed and, as a direct result, the conformational freedom has been shown to be altered.

The effect of solvent on the flexibility is evident from both models of solvation. Whereas the influence of DMSO, calculated within the frame of the continuum approach and shown in Figure 5a, is only moderate, with slight enlargement of the region bordered by the 1 kcal mol^{-1} contour, the influence of the water is significant (Figure 5b) and can be characterized as a considerable shrinking of the conformational region in which maltose should reside, at the considered temperature. A similar conclusion can be drawn from molecular dynamics simulation on the long-range time scale (Figure 6). The allowed region, where the molecule fluctuates, is smaller for both simulation (300 and 400 K) temperature in comparison to the corresponding vacuum simulation. The influence of the temperature on conformational sampling seems to be more important than it is in the vacuum simulation. The increase of the simulation temperature to 400 K (Figure 6b) does not allow a similar exploration of the conformational regions as seen in previous vacuum simulations even at lower temperature of 300 K (Figure 4a). This result contradicts some previously published results of MD simulations in solvent, where the authors conclude larger flexibility in water than in vacuum [11]. In our opinion the opposite applies. We believe that a consistently parametrized force field will not overestimate stabilization via intramolecular hydrogen bonding. This means that during the vacuum simulations the internal flexibility of carbohydrates may not necessarily be damped. On the other hand in solvent the internal flexibility is reduced due to environmental effects (friction of solvent molecules) which can be considered as a significant force operating against the frequent conformational jumps of the solute molecule.

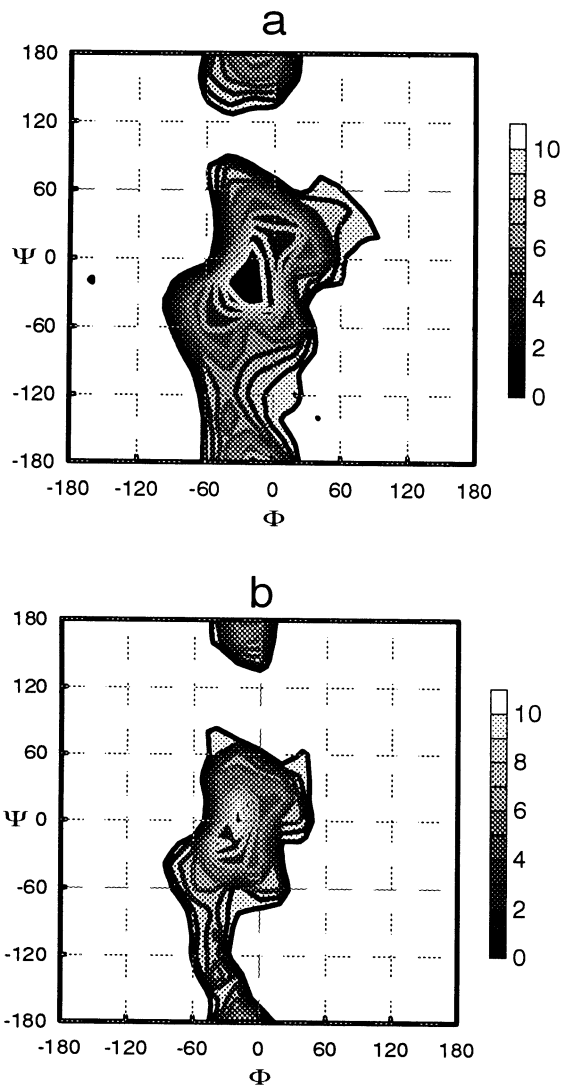

Figure 5. Influence of solvent on potential energy profile of maltose calculated with continuum model (a) Dimethyl sulfoxide (DMSO) (b) Water.

Damping of the conformational movement has also been observed during the 1 ns molecular dynamics simulation of maltose in the DMSO environment (Figure 7). Besides significant decrease of the conformational flexibility strong preference of the A conformational region, located around −60/−40° has been observed. The time-averaged virtual conformations from these simulations are −54.9/−36.3 at

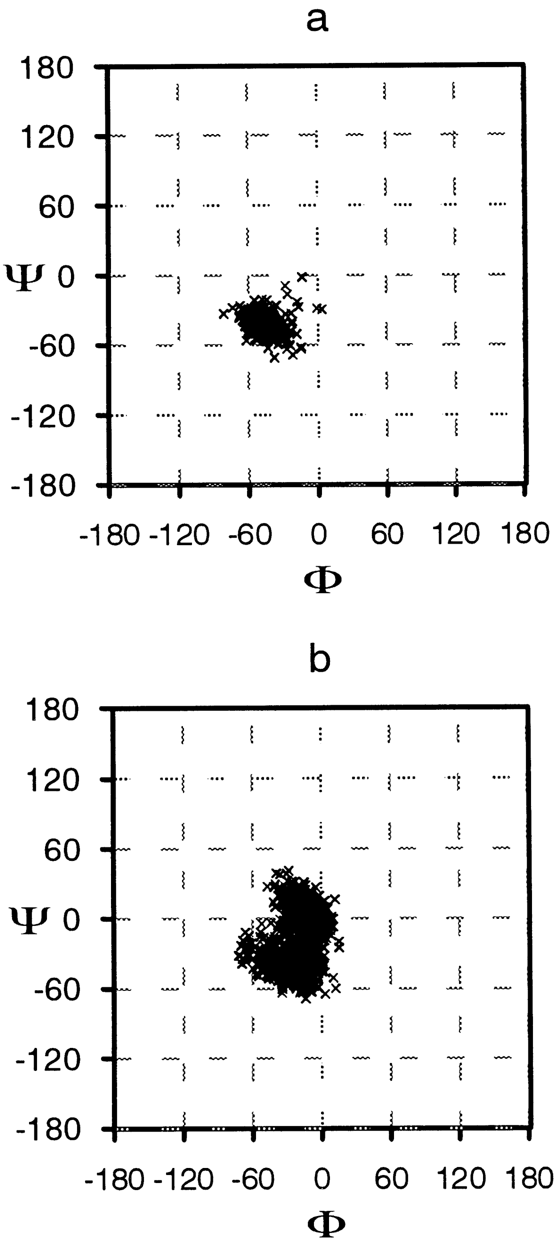

Figure 6. Effect of water on *Φ*, *Ψ* profiles of maltose as seen from molecular dynamics simulation (a) 270 ps simulation at 300 K (b) 910 ps simulation at 400 K. Dielectric constant 1 and 1 fs integration step has been used in both simulations.

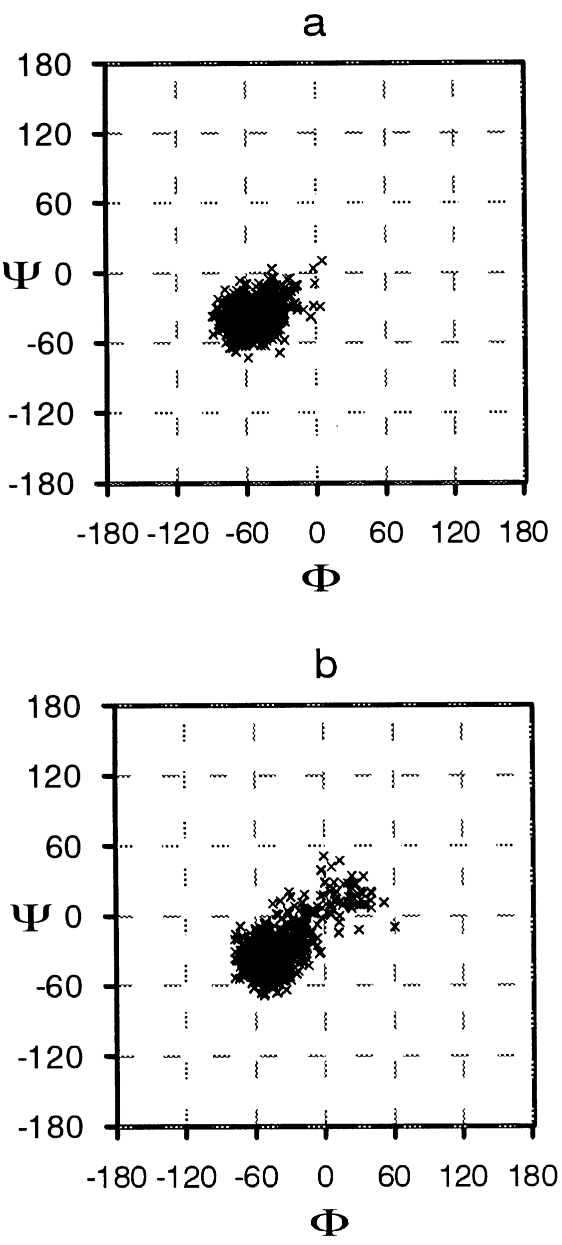

Figure 7. Influence of DMSO on *Φ*, *Ψ* profiles of maltose as seen from molecular dynamics simulation at 300 K (a) and 400 K (b). Dielectric constant 1 and 1 fs integration step has been used in both 1.05 ns simulations.

300 K and −45.2/−31.6 at 400 K, showing only a small influence of the simulation temperature on the averaged *Φ*, *Ψ* torsional angles. Based on experimental data, Stevens [30] concludes that the predominance of this conformational region should be realistic.

One can argue that relatively good agreement between the RAMM potential energy surface vs. CVFF molecular dynamics simulation and continuum vs. discrete solvent models might be accidental and valid only for the sample case of maltose. To test all of the above methods, *ie* how precisely they can estimate the conformational preference of other oligosaccharides, connected with different glycosidic linkage, a modelling study on the conformational preference of α-L-Fuc(1-2)β-D-Glc-1OMe has also been carried out and the discussion of the results follows.

The influence of the water environment on the occupancy of the 2D-*Φ*, *Ψ* conformational diagrams (for vacuum and water diagrams see Figure 8) is immediately evident and independent of the fact that the water simulation approaches only two-thirds of the length vacuum simulation.

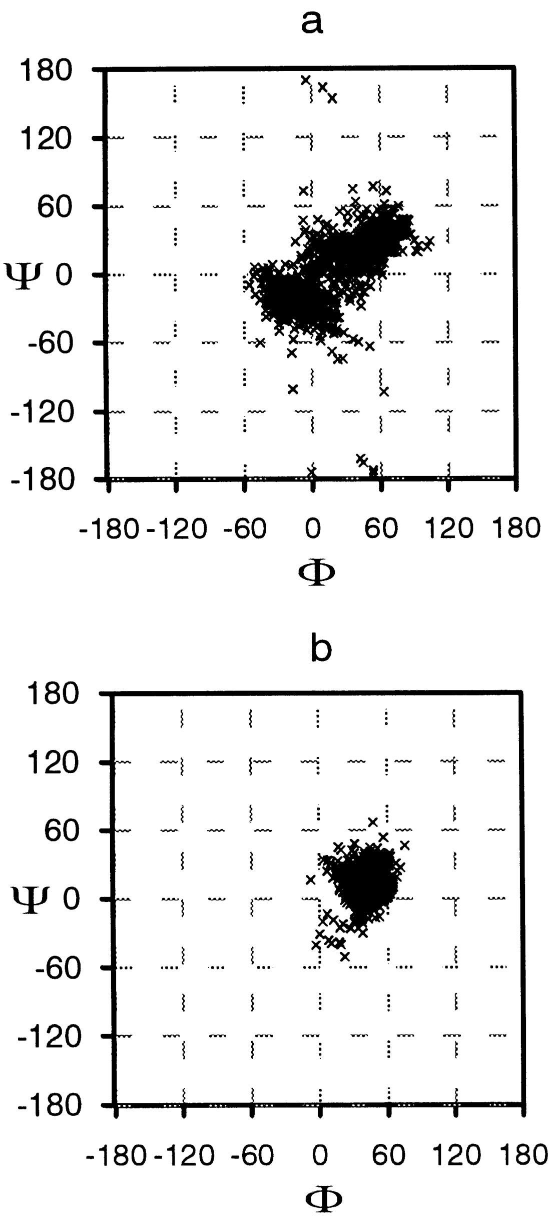

Figure 8. (a) Vacuum (1050 ps) and (b) water (640 ps) molecular dynamics simulations of α-L-Fuc(1–2)β-D-Gal-1OMe. The simulation parameters were as follows: 300 K simulation temperature, 1 fs integration step, 50 ps equilibration and dielectric constant ε equal 1.

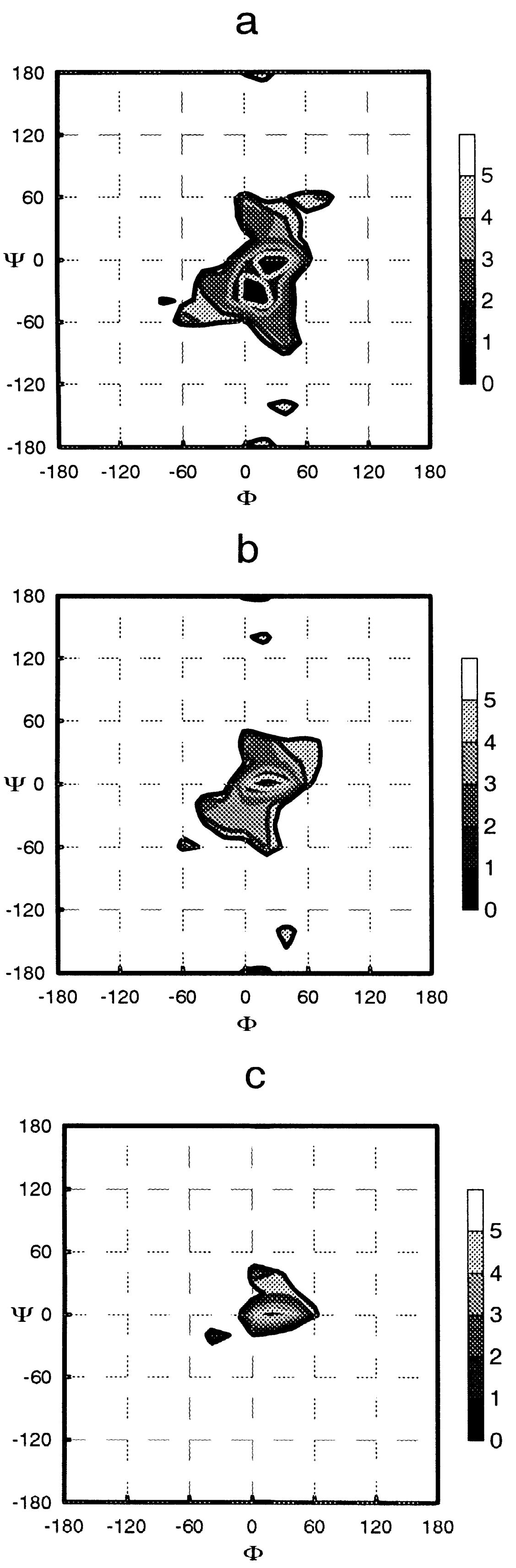

Figure 9. Solvent effect based on continuum approach. The calculated conformational energy profiles of α L-Fuc(1-2)β-D-Gal-1OMe up to 5 kcamol^{-1} above the global energy minimum are shown. (a) Vacuum calculation with $\varepsilon = 1$ used for electrostatic energy term. (b) Calculation in dimethyl sulfoxide (DMSO). (c) Water.

The sequence of the RAMM conformational energy profiles (for better visibility only the energy contours up to 5 kca mol^{-1} are drawn) for vacuum (Figure 9a), DMSO (Figure 9b), and water (Figure 9c), leads to a similar conclusion: the solvent, especially water, dramatically decreases the conformational freedom of α-L-Fuc(1-2)β-D-Glc-1OMe.

4. Conclusions

The two methods for approaching the solvent effect (discrete and continuum) resulted in a very similar observation of considerable damping of the conformational freedom within the Φ, Ψ space as a consequence of the bulk environment. In spite of the encouraging agreement of the presented solvation models, additional work is required to improve the modelling protocols, taking into account all of the approximations behind the solvent effect calculation and the shortcomings of the parametrizations of the force fields used in this study. The continuum model can certainly be amended by better calculation of the conformational dependence of the atomic point-charge distribution of the solute molecule in order to obtain a better description of the solute-solvent electrostatic interaction terms. Similarly, the conformation dependence of the atomic charges should also be considered within the frame of the molecular dynamics simulations, where, in the recent calculations, charge sets of constant values are used. Molecular dynamics simulations in solvents should be done over larger simulation time scales. Most importantly new solvation models combining the advantages of the continuum/discrete approaches (*eg* time efficiency of the first and realistic description of the specific solute–solvent interactions, as hydrogen bonding, of the second) should be developed and tested for prediction of conformational behaviour of carbohydrates and other biomolecules and compared with experimental results.

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