Recent Trends in Carbohydrate Modeling

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Abstract: The exploding activities in modeling of carbohydrates during the past few years is reviewed with emphasis on advances in improving force fields, coupling of NMR measurements with molecular dynamics simulations, direct calculation of thermodynamic properties, application of quantum chemical methods on a large scale, and web-access to modeling.

1. INTRODUCTION

When modeling of chemical substances of biological importance began, peptides was the first choice, due to their relatively small size and well-defined geometry. Since about 1980, increasing attention has been given to carbohydrates. The late start is due to the fact that carbohydrates are extremely flexible, so that molecular mechanics methods (Molecular Statics, MS, and Molecular Dynamics, MD) could not be properly applied until adequate software and faster computers were available.

The past decade has seen an explosion in applications of modeling methods to carbohydrates. This phase is described in reviews by, among others, French, and Imberty and Perez. French [1] reviewed the entire subject of carbohydrates, in a report on all important aspects of modeling. Imberty and Perez [2] reviewed extensively recent progress in conformational studies of bioactive oligosaccharides by modeling and by NMR, with a view to the design of ligands for protein binding and, as a distant goal, carbohydrate-based drugs.

The present review deals with the latest trends along the following lines: development of force fields specifically suitable for carbohydrates for applications in MS and MD; extensive MD studies, mostly coupled with NMR measurements; hybrid MS and *ab initio* studies; large-scale *ab initio* computations; application of modeling methods to thermodynamic properties, and on-line access to modeling. Some of these trends were touched on in a meeting on three-dimensional structures of carbohydrates and how they interact with proteins. An entire issue [3] was devoted to contributions from this meeting.

2. FORCE FIELD DEVELOPMENT

Woods [4] contributed an extensive review of essentially all force fields available at the time of writing, which is a must for those wanting to go into the field. Unfortunately, this has not been updated. This is now a heavy task which ought to be undertaken by broad cooperation by all developers of force fields. The first initiative in this direction was taken by Perez and Imberty [5] who organized a comparison of some force fields, distributing a set of structures for the participants to study. The answers were treated by statistical methods. This initiative ought to be extended, with respect to force fields, range of compounds, and range of properties. Such an undertaking would merit an extensive review, with a detailed comparison of force fields, their energy functions, parameter sets, and results of their application.

As just indicated, an extensive review of all recent developments is a formidable task, and it is out of the question for the present purpose; rather, current trends are highlighted. Two trends have been obvious: many attempts are made to improve the established force fields, and to include more functional groups.

Ragazzi and coworkers [6] include the ionic groups sulfate and carboxylate with their own version of MM2, using *ab initio* methods which take a substantial part of electron correlation into account. Carboxylate groups are added to the Biosym CVFF force field by Miertus *et al.* [7] in a slightly less sophisticated approach. Sulfate groups were added to the Tripos force field by Perez and coworkers [8] using x-ray data. The SPASIBA force field which is derived from the AMBER by addition of typical spectroscopic terms, was perfected specifically for glucose by Vergoten and coworkers [9]. Such a complicated force field will contain many correlated force constants. The procedure can be dangerous as unnecessary fudge-factors may be introduced, and eventually the transparency of the PEF is lost. Spieser *et al.* [10] from the Kroon group have improved the GROMOS force field for saccharides. Glennon and Merz devised an AMBER force field for saccharides [11] by a combined *ab initio* - MS procedure. They claimed that it is optimized, but this statement is not true in the strict sense, as no objective computational procedure is applied as, e. g., in the CFF approach [12].

An extension of CHARMM22 was made by Kouwijzer and Grootenhuis [13] who in CHEAT used extended atoms which means that hydroxyl hydrogen atoms are imbedded in the oxygen atoms they reside on. The purpose of this approach is to be able to perform computations on molecular systems with a larger number of atoms.

For force fields based entirely on *ab initio* procedures, see below.

3. MOLECULAR DYNAMICS

In MD simulations, Newton's equations of motions are solved; the time-dependent motion of particles is thus

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obtained by MD simulations. This facilitates computation of thermodynamic properties of the system, as density, heat capacity, free energies among others for a large number of carbohydrate conformers.

A major advantage of MD simulations, compared to gas phase QM and MS calculations, is that solvents can be treated explicitly. Previously, water was treated implicitly using a model for the solvent, but increasing computer capabilities allow explicit inclusion of water. This means that solvated systems can be modeled and thermodynamic properties calculated at a specified temperature and pressure. Furthermore, very important protein-carbohydrate interactions can be modeled under realistic physiological conditions.

A crucial point to this is, however, that an appropriate force field is available. Effort has therefore been made toward developing suitable parameters for carbohydrate force fields, as previously mentioned, and verification of these force fields.

Simulation of glass transition temperatures have been carried out for different concentrations of an aqueous solution of glucose has successfully been carried out by Grigera et. al. [14]. The melting point was also investigated in this study, however, these results were not as good as those obtained for the glass transition temperature.

Jimenez-Barbero *et al.* [15] have studied the solution properties *in vacuo* of methyl α-lactoside, with each of the three force fields Amber/Homans [16], CVFF [17] and CFF91 [18], using dielectric constants of both ε=1 and ε=80 Debye, respectively, to represent the solvent. Upon comparison of MS and MD simulations with experimentally measured steady state and transient NMR NOEs (Nuclear Overhauser Effects), they found that the general purpose CVFF force field gave results having the best qualitative agreement with experiment.

A similar study has later been carried out by the same group [19] using two other force fields instead, the MM3 [20] and the ESFF [21] force field. This study showed that the MM3 force field was superior to the ESFF force field. Results obtained using the ESFF force field were in qualitative agreement with experiment, whereas MM3 reproduced the experimentally measured solution properties of several methyl α-lactoside conformations well.

Presently, conformational studies of carbohydrates is most often carried out as a combination of MS and MD calculations, and experimental and calculated NMR NOE data. This provides a very strong tool for an elaborate study of carbohydrate flexibility. By MS calculations a detailed picture of a part of the energy surface as a function of the glycosidic torsional angles can be obtained for disaccharides and oligosaccharides. The behavior of the glycosidic bonds and the hydrogen bond pattern in vacuo and in solution may then be studied with MD. The predicted low energy conformations can be verified against experimentally measured NMR data, which represent a time-averaged dynamic ensemble of conformers.

Conformational analysis using these methodologies have been carried by Hervé du Penhoat *et al.* [22] for four disaccharides, αGalA-(1→4)- αGalA, ethyl-β-Gal*p*--(1→4)-β-Glc*p*, trichloro-4,1′,6′-trideoxy-α-gal*p*-(1→2)- β-Fru*f* and finally α-Ara*f*(1→5)-α-Ara*f* in order to elucidate the internal motion of carbohydrates in vacuo. These calculations were performed using CHARMm [23] with a force field modified for carbohydrates [24], applying the SHAKE [25] algorithm to keep bond lengths fixed during simulation.

Ethyl-β-lactoside has also been investigated by Penhoat et. al [26], in a study of three different force fields. The force fields are based on the MM3 [20], CFF[27], and CHARMm [23] , all modified for carbohydrates. This study concluded that in particular calculated NMR data varies a lot depending on the chosen force field.

The conformational preference of the cyclic system cyclosophoroheptadecaose, consisting of 17-24 glucosides in (1→2)- β-glucan units, have been studied by Tvaroska *et al.* [28], in order to elucidate the ability to form inclusion complexes with other compounds. The lowest energy conformers were identified by variation of the glucosidic torsional angles with MS using the MM3 force field in the CHARMm [23] program. The trajectories of the conformers were subsequently computed by MD. ^{n}J values were obtained from the simulation as well as Karplus relations, and were compared to experimental NMR data, eventually leading to the conclusion that cyclosophoroheptadecaose only to a small degree is capable of forming inclusion complexes as previously anticipated.

Widmalm *et al.* [29] has studied the disaccharide α-L-Rha*p*-(1→2) α-L-Rha*p*-(1→OMe) using several force fields [16, 23-24, 30], and varying the dielectic constant ε, as they found that MD simulations sensitive to this. The conclusion of the study was that gas phase optimized force field parameters may have to be modified when used in MD simulations in which water is treated explicitly.

A study of GlcNAcβ-(1→4)GlcNAcβ-OMe by Aida *et al.* [31] also confirmed the sensitivity of MD simulations on the force field as they obtained different dominant conformations. The change in conformation when water is treated explicitly is caused by formation of intermolecular hydrogen bonds compared to having only intramolecular bonds when solvent is not present, and the molecule is treated in a continuum, characterized by the dielectric constant.

The importance of explicit inclusion of water has also been emphasized by Kirschner and Woods [32] in a study of the rotamer distributions about the glucosidic torsional angles in disaccharides. Only the presence of water creates a hydrogen bond pattern which reproduces the experimental rotamer populations correctly.

These examples show that a lot of effort is put into conformational analysis of carbohydrates, using MD, aided by MS calculations and NMR data. However, caution should be taken, especially toward force field and methodology used for description of the solvent.

As a consequence of more powerful computers, other simulation methods have evolved, allowing calculation of carbohydrate-protein interactions and other problems of interest. One such method is the Free- Energy Perturbation (FEP) method, which accounts for both entropic and enthalpic energy contributions to the binding energy [33].

Here, the free energy of a ligand in its bound state is calculated and compared to its free state in solution. This procedure enables direct comparison of preferred binding of different ligands to proteins, therefore, it is a very useful tool for prediction of binding of specific ligands to specific receptors, which is important in medicinal chemistry.

4. MODELING THERMODYNAMIC PROPERTIES

Jónsdóttir has devised a procedure for modeling phase equilibria based on the UNIQUAC and UNIFAC models by the use of non-bonded energies from MS calculations. In these thermodynamic models interaction coefficients for a nonbonded complex, consisting of two molecules, is obtained by calculation of the energies of each of the complexes. The complexes are carbohydrate-carbohydrate, water-water and water-carbohydrate, respectively. The conformational space and sampling of significant conformations of the complexes are, prior to energy minimization, investigated by the Boltzman Jump Procedure. The UNIQUAC interaction energies can be computed from these energies and subsequently used for calculation of thermodynamic vapor-liquid equilibrium and solid-liquid equilibrium data. This rather unique approach has seen success for several classes of substances and has lately been applied to vapor pressures of aqueous solutions of carbohydrates [34] and to the solubility of carbohydrates in water [35].

Another approach to estimate thermodynamic properties of carbohydrates is given by Dyekjær *et al.* [36]. Here QSPR (Quantitative Structure-Property Relationships), i.e. linear relationships between the property of interest and molecular parameters depending on each individual molecular conformer are developed. These so-called molecular descriptors are obtained from MS and DFT calculations, and conformational flexibility is accounted for by systematically generating and selecting significant conformations. Based on this methodology relationships between several monosaccharide structures and the physical properties melting point, glass transition temperature, density, heat of fusion, heat of vaporization and partial molar heat capacity are found. However, when applying the models to predict these properties for the more conformationally complicated disaccharides, only estimates for density and partial molar heat capacity are successful. The deficiencies seen when modeling solid state properties is explained to be caused by lack of the fairly simple models to properly reproduce effects arising from the crystal lattice.

5. HYBRID MS - QUANTUM-CHEMICAL METHODS

It has long been a wish to apply quantum chemical methods to modeling. In the case of carbohydrates it is particularly difficult, as the relevant molecules characteristically contain upwards of 24 atoms, half of them carbon and oxygen. Reasonable results require large basis sets and computations carried out at a high level of theory, where electron correlation is accounted for. Many of the necessary prerequisites have become available only within the past decade, and although it is now quite feasible to calculate reliable equilibrium structures of monosaccharides, it still not commonplace work to perform large *ab initio* calculations on disaccharides and higher oligosaccharides.

In order to avoid the largest and heaviest calculations, and also to improve modeling with MM3, French and his coworkers developed an approach in which they calculate structures and energies of carbohydrate analogs, which are carbohydrates stripped of alcohol groups, with *ab initio* procedures and with MS using MM3, and use the differences to scale MM3 calculations of the entire carbohydrates.

Their first paper [37] describes the procedure in detail, and its application to Ramachandran plots of the potential energy of three disaccharides as a function of the two torsional angles of the glycosidic linkage. If such detailed plots are desired, calculation by *ab initio* methods are still out of question as an everyday routine, and modeling is the only possibility. The energy-correction method of French and coworkers is a temporary answer.

In another paper [38], the same group calculates energy surfaces for a number of disaccharide analogs using HF/6-31G*. One might ask whether HF, and a rather restricted basis set, is an adequate model for molecules with lone pairs and in situations where considerations of non-bonded interactions demand that the greater part of the electron correlation be taken into consideration.

A further attempt to improve MM3 for carbohydrates has been made in a calculation of heats of formation of disaccharide analogs [39].

6. PURE QUANTUM-CHEMICAL METHODS

Momany [40-41] has begun a monumental project of computing structures and energies of many conformers of disaccharides using an ambitious level of theory, B3LYP/6-311++G** and full geometry optimization. The AMBER force field is modified to reproduce the results [42]. The methods are applied to studies of internal hydrogen bonding in disaccharides [43] and to solution studies of cyclic oligosaccharides [44].

Similar work has been done before, notably by Woods *et al.* who developed the parameter set GLYCAM for AMBER [45]. The work is based on a lower level of theory applied to one monosaccharide and a number of analogs. It was close to the limit of what was technically feasible at the time, and the outcome was a success for the purpose, MD modeling of the behavior of the glycosidic linkage in methyl-glucopyranosides and methyl-mannopyranosides.

Jorgensen and coworkers [46] fitted their OPLS force field to *ab initio* calculations on monosaccharides, using RHF/6-31G* for geometry optimization and B3LYP/6-311+G* for energies.

Reiling *et al.* [47] constructed a new parameter set for CHARMM22 [48] from MP2/6-311+G** energy calculations of HF/6-31G** geometry optimized “fragment” molecules like methoxymethanol, methoxyethanol and dimethoxymethane.

The CVFF force field which is developed in a rather special procedure [49] was extended to carbohydrates [50].

Also Tvaroska and coworkers used *ab initio* methods during a number of years. A recent example is a study [51] of conformations of the hydroxymethyl and methoxymethyl groups in aldohexosides at levels of theory up to B3LYP/6-311++G**.

A new force field for carbohydrates is presently being optimized by Rasmussen [52] based on a large number of model compounds including alkanes, ethers, alcohols and saccharides, following a previously developed strategy [12]. The *ab initio* calculations are done exclusively on the B3LYP/6-311++G** level using PQS running on a Quantum Station [53]. The CFF optimization is made on structure, but now also also on binding energy which, in the MS sense, is the molecular potential energy. In a subsequent refinement, it is the intention to include data from Momany's work in the optimization.

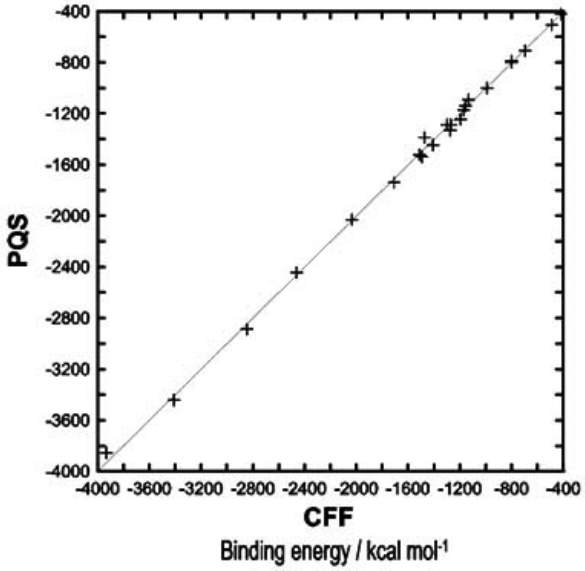

Fig. (1). Binding energies calculated with an *ab initio* and an MS method.

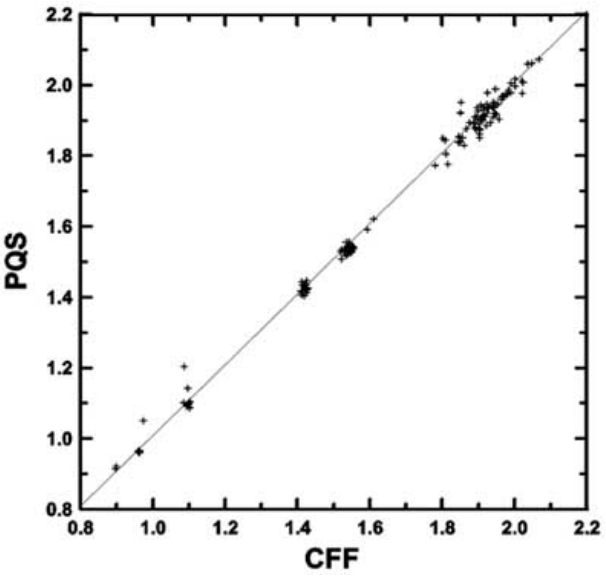

Fig. (2). Geometric data calculated with an *ab initio* and an MS method.

Figs. **1** and **2** will give indication of the precision which can be obtained with the latest methods. They stem from recent work and have not been published elsewhere.

7. ON-LINE MODELING

Von der Lieth and his group have developed the softwares SWEET [54] and SWEET-DB [55]. SWEET is a web-based tool [56] for conversion of carbohydrate sequence information into 3D models which can be visualized and written to files in several different file formats [57]. SWEET-DB is a tool for building SWEET-DB databases [58]. For these purposes they developed [59-60] LINUCS: LInear Notation for Unique description of Carbohydrate Sequences. The system is being further developed with on-line modeling; simulation is done on a Linux cluster using TINKER and, at present, AMBER, MM3 and OPLS-AA. More will undoubtedly be added.

This interactive capability may well be the answer to many groups who do not want to toil with their own development or adaptation of models.

ABBREVIATIONS

6-31G*, 6-311+G*, 6-311++G**	=	different basis sets for HF, RHF
AMBER	=	Assisted Model Building and Energy Refinement
B3LYP	=	Special functional in DFT
CFF	=	Consistent Force Field (Lifson *et al.*, Rasmussen *et al.*)
CFF91	=	Consistent Force Field (Hagler *et al.*)
CHARMM	=	Chemistry At Harvard Molecular Mechanics
CHARMm	=	Chemistry At Harvard Molecular Mechanics commercial version
CHEAT	=	Carbohydrate Hydroxyl groups represented by Extended AToms
CVFF	=	Consistent Valence Force Field (Hagler *et al.)*
DFT	=	Density Functional Theory
ESFF	=	Extensible Systematic Force Field
FEP	=	Free Energy Perturbation
GLYCAM	=	GLycosides and glycoproteins with AMBER
GROMOS	=	GROningen MOlecular Simulation
HF	=	Hartree-Fock
LINUCS	=	LInear Notation for Unique description of Carbohydrate Sequences
MD	=	Molecular Dynamics

MM	=	Molecular Mechanics
MM2, MM3	=	Molecular Mechanics (Allinger *et al.*)
MS	=	Molecular Statics
NOE	=	Nuclear Overhauser Enhancement
OPLS	=	Optimized Potential for Liquid Simulations
OPLS-AA	=	Optimized Potential for Liquid Simulations – All Atoms
PEF	=	Potential Energy Function
PQS	=	Parallel Quantum Solutions
QM	=	Quantum Mechanics
RHF	=	Restricted Hartree-Fock
UNIFAC	=	UNIversal quasichemical Functional group Activity Coefficient
UNIQUAC	=	UNIversal QUAsiChemical

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