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Restrained electrostatic potential atomic partial charges for condensed-phase simulations of carbohydrates*

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Abstract

Charges derived from fitting a classical Coulomb model to quantum mechanical molecular electrostatic potentials (so called ESP-charges) are frequently used in simulations of macromolecules. Simulation methods that use ESP-charges generally reproduce the geometries of hydrogen bonded complexes, despite the fact that these charges are known to overestimate the strengths of these interactions. Through the use of a restraint function during the fitting of the partial charges to the electrostatic potentials the magnitudes of the charges may be attenuated (so called RESP-charges). For the AMBER force field RESP-charges have been proposed for proteins and nucleic acids. Here we examine a novel approach for determining the RESP-charges for carbohydrates based on molecular dynamics (MD) simulations of crystal structures. During a simulation, the crystallographic unit cell geometry is sensitive to both inter-molecular non-bonded forces and internal torsional rotations. However, for polar molecules, and specifically carbohydrates, the crystal geometries are particularly sensitive to the set of partial atomic charges employed in the simulation. Thus, given a force field in which the van der Waals and torsion terms are well parameterized, it is possible to assess the suitability of a set of partial charges by monitoring the properties of the crystal during an MD simulation. We have examined several charge sets for use with the GLYCAM parameters for carbohydrate and glycoprotein simulations and found that a restraint weight of 0.01 gives the best agreement with the neutron diffraction structure of α -D-glucopyranose. Unrestrained ESP-charges performed poorly as did the charges obtained from Mulliken and distributed multipole analyses of the quantum mechanical HF/6-31G* wavefunctions.

Keywords

AMBER; Carbohydrate; Electrostatic potential; GLYCAM; Molecular dynamics simulations; Restrained electrostatic charges

1. Introduction

Most macromolecular force fields employ atomic partial charges when computing electrostatic forces and energies. These forces are particularly important in biomolecules, in

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which the strengths of hydrogen bonds are largely determined by the electrostatics of these polar interactions. Accurate treatment of electrostatic interactions is essential if structural features such as α -helices and β -sheets in proteins are to be correctly maintained during the course of a molecular dynamics (MD) simulation. In addition to the case of these relatively rigid structural elements, electrostatic forces may also influence dynamic motions. For example, the frequently observed dynamic behavior of carbohydrates in a solution depends on a subtle balance of forces between intramolecular hydrogen bonds and those between the solute and the solvent [1]. The structures and energies of carbohydrate-protein complexes are similarly sensitive to subtle differences in electrostatics between the free and bound states [2].

Currently, it is a common practice to deduce a set of atomic partial charges for a single conformation of the molecule. For relatively rigid molecules this approach is valid; however, for flexible molecules attention must be directed also to the effect of conformation on partial charge [3,4]. A further limitation of current simulation methods is that the charges cannot respond to changes in the molecular environment. While charge polarizability is, in principle, possible to include in a simulation [5], it remains one of the most challenging areas of the force field parameter development.

Despite the general utility of atomic partial charges, the partial charge model is artificial and consequently there is no single “correct” method for charge derivation. There are, however, certain desirable properties for any charge set, including the need to reproduce inter- and intramolecular energies, display sensitivity to molecular conformation and configuration, and respond to external environment. Partial charges (q) derived from optimizing the fit (χ_{esp}^2) between the classical model for the electrostatic potential ($\hat{v}$) and the quantum mechanical molecular electrostatic potential (V) evaluated at points (i) around the molecule (so called ESP-charges) are frequently employed in MD simulations.

$$\chi_{esp}^2 = \sum_i (V_i - \hat{V}_i)^2, \quad (1)$$

where

$$\hat{V}_i = \sum_j \frac{q_j}{r_{ij}} \quad (2)$$

Alternative approaches to deriving atomic partial charges include empirical fitting to experimental properties (heats of vaporization or sublimation, liquid densities, or gas-phase dipole moments) [6,7] and partitioning of quantum mechanical wavefunctions (Mulliken, Bader and distributed multipole analyses) [8,9].

The ESP-charges computed with a 6-31G* basis set provide a reasonable description of the electrostatic properties of many polar molecules, yet are known to overestimate bond polarities in the gas-phase [3]. The most common justification for the continued use of 6-31G* ESP-charges is that their enhanced bond polarities, especially for water, are suitable for condensed phase simulations in which polarization is expected to be present. However,

as Bayly et al. [3] have noted, there is no reason to expect that the solute should be as polarized as the solvent. This is particularly significant in the case of carbohydrate simulations, in which there exists a delicate balance between the energies associated with water–sugar interactions and internal sugar–sugar interactions. Both interactions involve OH–OH hydrogen bonds, and an inaccurate charge model could artificially swing the balance in favor of one over the other. Consequently, the protocols for deriving atomic partial charges for proteins are not necessarily suitable for carbohydrates. In the development of the GLYCAM parameters for simulations of solvated carbohydrates [10], we employed ESP-charges. These charges have performed well in simulations of carbohydrate structures in a solution as indicated by comparison with the experimental NMR data [1,11]. However, we wished to extend our studies to the binding energies of complexes of carbohydrates and proteins [2], and concerns over excessive polarization of the carbohydrate prompted us to reconsider the choice of charge protocol.

The bond polarity may be attenuated by the inclusion of a hyperbolic restraint function (χ_{rstr}^2) during the fitting the classical potential to the quantum electrostatic potentials, which serves to lower the magnitude of the charges (RESP-charges).

$$\chi_{resp}^2 = \chi_{esp}^2 + \chi_{rstr}^2, \quad (3)$$

where

$$\chi_{rstr}^2 = k_{rstr} \sum_j \left((q_j^2 + b^2)^{1/2} - b \right) \quad (4)$$

The tightness of the hyperbola around its minimum is determined by b , while the strength of the restraint function is determined by the restraint weight k_{rstr} . An advantage of this method over alternatives, such as empirical charge scaling, is that the RESP-fitting does not greatly affect the molecular dipole moments and maintains an integral net charge on ions. Restraint weights of 0.001–0.01 have been proposed for calculating partial charges for polar molecules in general [3,4], and in this paper we examine the influence of k_{rstr} on MD simulations of a representative carbohydrate, α -D-glucopyranose. In many instances RESP-fitting is performed in a two-stage process [3,4] that enables the charges on hydrogen atoms to be adjusted to reflect their molecular or rotational symmetry after an initial charge fitting has been performed. In carbohydrates, neither molecular nor rotational symmetry is present and a single stage fitting is appropriate.

To validate the RESP-charges for use in the condensed-phase simulations, we believe simulations of a carbohydrate crystal offer several advantages over simulations of a carbohydrate in solution. The most obvious advantage is the precise nature of the experimental structure. In contrast, a solvated carbohydrate frequently exists in several conformational states, the populations and precise structures of which cannot be generally verified experimentally. Further, in order to obtain an accurate conformational ensemble for a carbohydrate in solution at least 1000 ps of simulation time is required. In the crystal, the atomic motions are greatly reduced and adequate sampling is achieved in approximately 50

ps. Lastly, the crystal model offers a clear choice of a single conformation for which to compute the partial charges. Although a crystalline environment is not identical to that of a solvated molecule, this difference is less significant for carbohydrates. Carbohydrates in the crystal state always exhibit extensive OH–OH intermolecular interactions [12], which are similar to those present between a carbohydrate and water molecules in solution.

The focus of this paper is to derive a set of partial charges for condensed-phase simulations of carbohydrates that allows the highest possible accuracy within a static (non-polarizable) RESP-charge model.

2. Computational methods

Electrostatic potentials were computed at the HF/631G* level at points around the surface of the molecule as determined using the CHELPG option of Gaussian 94 [13]. Distributed multipole analyses were performed with CADPAC [14]. Crystal simulations were performed with amber 4.1 [15] as implemented in the Discover 3 module of InsightII version 950 from Molecular Simulations [16]. The Discover simulations employed a version of the GLYCAM parameter set [10] converted from AMBER format for application with Discover 3. MD simulations were performed on a Silicon Graphics R10000 Indigo2. In order to ensure that the motions of each molecule were sensitive only to the force field, we elected not to impose crystallographic symmetry during the simulations. Following the model proposed by Kouwijzer et al. [17], we created a crystal that contained 64 molecules (Fig 1). This structure corresponded to a simulation box containing $2 \times 2 \times 4$ crystallographic unit cells [18] and ensured that the use of a 10.5 Å cut-off for non-bonded interactions did not introduce imaging artifacts. The simulations were performed under periodic boundary conditions with coupling to an external temperature bath at 298 K and coupling to a pressure bath at 1 atm. A time step of 1 fs was employed during the simulations, which were performed with a constant dielectric of 1. No constraints were placed on the bond lengths. The simulations were initiated from the neutron diffraction structure for α -D-glucopyranose, which exists in an orthorhombic ($P2_12_12_1$) unit cell [18]. The experimental geometry for the monosaccharide [18] was employed in all quantum mechanical calculations. The RESP calculations [15] were performed with a hyperbolic restraint function (Eq. (4)) [3,4] and the fitting was carried out in a single-stage to the electrostatic potentials at 13670 grid points generated by CHELPG.

3. Results and discussion

The MD simulations were performed for 50 ps total time and of that only the last 30 ps were included in our analysis. In agreement with the findings of Kouwijzer et al. [17], during the first 20 ps the simulations reached equilibrium, as determined both from the temperatures that had stabilized at 298 ± 1.5 K over this period, and from the energies that had reached equilibrium within that period.

Table 1 presents the average crystal dimensions and energies for a series of simulations performed with several charge sets. The results from the simulations could be divided into one of three categories: (A) the experimental crystal geometry and intermolecular

interactions were reasonably maintained; (B) the intermolecular hydrogen bonds were maintained but the unit cell geometry was distorted; or (C) neither cell geometry nor interactions were maintained.

When unrestrained ESP-charges ($k_{\text{rstr}} = 0.0$) or only weakly charges ($k_{\text{rstr}} = 0.005$) were employed in the simulations, the crystals distorted spontaneously from the experimental structure. In the distorted structure (the B-structure), the basic network of hydrogen bonds was largely retained, however, slight translations of the individual molecules led to alterations in the unit cell lengths (see Table 2). This distorted structure was seen to generate a unit cell in which the a and b dimensions were significantly decreased (by 5–6%) while the c dimension expanded by 8–9%, resulting in an overall volume decrease of 2–2.5% (Fig. 2). In contrast, when restraint weights of 0.01 or larger were employed, the individual molecular orientations were maintained and the simulations led to good agreement with the experiment. Notably, when a uniform scaling of 0.9 was applied to the unrestrained ESP-charges, so as to lower their magnitudes (see Table 3), the crystals spontaneously distorted to a disorganized structure (C), in which the internal hydrogen bonding connectivities and symmetry were not maintained. Empirical scaling by 0.9, applied to the RESP ($k_{\text{rstr}} = 0.01$) charges, did not result in the crystal distorting from the A-geometry. Although the net volume change for the unit cell (–0.7%) obtained with the scaled RESP-charges was better than that for the other charge sets, the average change in cell lengths (1.4%) was comparable to that obtained from the simulations performed with larger restraint weights. We believe that the disadvantages of empirical scaling, particularly when applied to ionic systems, severely detract from its general utility. In all simulations the average unit cell angles were found to be within 0.5° of 90° .

A further measure of the degree of distortion of the internal molecular orientations is provided by computing the root mean squared deviations (rmsd) in atomic position for the simulation, relative to the experimental structure. For the A-geometries, the average rmsd for the non-hydrogen atoms was 0.38 Å while in the B-geometry the rmsd was approximately 1.0 Å. These deviations clearly indicate that the B-structure is inconsistent with the experimental data. The corresponding rmsd values for the hydroxyl protons only were 0.47 and 1.04 Å, respectively. From these values it is reasonable to conclude that in the A-geometry the hydroxyl groups do not display significant rotational transitions.

In all cases the unit cells contracted by typically 2–2.5%. This contraction may reflect the fact that the simulation employed static charges in what can be expected to be a polarizing environment. However, other effects, such as the van der Waals forces and torsion parameters may be contributing. To determine the extent to which torsional motions were responsible for either the spontaneous transition to the B-geometry or for the changes in the unit cell geometry we repeated two representative simulations while restraining all of the torsion angles at their crystallographic values. These geometric restraints had no apparent effect on either the simulations performed without charge restraints ($k_{\text{rstr}} = 0.0$) or on those performed with restrained charges ($k_{\text{rstr}} = 0.02$). From these results, we concluded that the distortions were attributable solely to the non-bonded forces.

It is interesting to note that the average potential energies are all negative and that the magnitudes are proportional to the restraint weight. Since reducing the charge magnitudes would be expected to lead to weaker hydrogen bonds, this result suggests that the potential energy contains large repulsive energy contributions that are decreased as k_{rstr} increases. An exception to this trend is seen for the charge sets empirically scaled by 0.9. This scaling severely decreases the stability of the crystals, reducing the average potential energies by a factor of approximately 0.5.

The potential energies for the A-geometry could not be determined for the unrestrained charge simulations, due to their spontaneous deformation to the B-geometry. However, by initiating simulations from the B-geometry, we were able to determine the energies of the B-geometry for each value of k_{rstr} . In all cases, the B-geometry was found to be more stable than the A-geometry and the energy differences (approximately 20 kcal mol⁻¹) did not appear to be sensitive to the values of k_{rstr} . For values of k_{rstr} equal to or greater than 0.01 spontaneous transitions to the B-geometry no longer occurred, suggesting the emergence of a significant barrier between the A- and B-states. It is extremely likely that the stability of the B-state is an artifact. As such, the most logical source of the problem is in the treatment of the non-bonded interactions between the individual molecules. It is the more stable geometry in all cases, and therefore must arise from a feature shared by each simulation. All simulations employed the same set of van der Waals parameters and non-polarizable charges were used throughout. Either of these features may contribute to the unexpected stability of the B-geometry. Further, the use of a cut-off in the calculation of the electrostatic energies may be the underlying fault. The energy difference between the A- and B-geometries is relatively insensitive to charge set, which may suggest that it arises from the van der Waals forces. On a per atom basis, this energy difference is an extremely modest 0.013 kcal. We are exploring further the origin of this intriguing problem.

Table 1 presents the results from two non-ESP charge sets. A simulation was performed with charges obtained from a Mulliken population analysis of the 6-31G_p wavefunction (see Table 3). These charges led to the largest deformation of the unit cell and were clearly unsuitable. Mulliken charges are known to be generally poor at reproducing intermolecular interaction energies [3]. The second non-ESP charge set was computed from a multipole expansion of the same wavefunction. This expansion included atom centered monopoles, dipoles and quadrupoles (see Table 3). With sufficient terms, such an expansion can provide a complete description of the molecular electrostatic properties [19] and multipole expansions have been applied in studies of polar organic crystals[20]. Not unexpectedly, the data in Table 1 indicate that monopoles alone from such an expansion are insufficient to describe the intermolecular forces, as indicated by the distortion of the unit cell to the B-geometry. We are continuing to examine the influence of higher multipoles on crystal simulations.

4. Conclusions

The simulations reported here provide a fast and sensitive method for evaluating the suitability of any partial atomic charge set for application in condensed phase MD simulations of carbohydrates. In principle these simulations could be applied to any system

for which an accurate experimental structure was available. Distortions in the crystal cell dimensions were large for each of the charge sets not derived from fitting to quantum mechanical electrostatic potentials. The average absolute errors in cell length for the non-ESP charges varied from a 7–8%. In contrast the ESP-derived charge sets led to distortions of between 1.3 and 7.1%. The use of RESP charges reduced the distortions significantly, provided the RESP charge fitting was performed with a sufficiently large restraint (0.01 or larger). Notably, the total potential energy for the simulations was inversely proportional to the RESP restraint weight. The decreasing charge magnitudes associated with increasing restraint weight apparently lead to a reduction in electrostatic repulsions, thereby lowering the total potential energies. This result suggests that unrestrained ESP charges overestimate electrostatic repulsions in polar molecules, such as carbohydrates. Regardless of the charge set, all simulations led to an overall contraction of the crystal geometry, which was relatively insensitive to the partial charge set. The simulations indicated the presence of a second stable geometry for the crystal, in which the intermolecular interactions are maintained, but in which the unit cell is distorted. The surprising stability of this second geometry suggests need for further improvement in van der Waals parameters or possibly the need to employ a polarizable charge model. Based on this work we are incorporating RESP-charges ($k_{\text{rstr}} = 0.01$) into the GLYCAM force field.

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Fig. 1. Simulated crystal lattice containing 64 molecules of α -D-glucopyranose. Carbon atoms are shaded in light gray, oxygen atoms in dark gray, unit cell translations are labeled **a**, **b**, and **c**.

Fig. 2.
Detail of non-bonded interactions between molecules in the crystal. A solid black line indicates potential hydrogen bond interactions. Labels refer to data in Table 2.

Table 1
Crystal unit cell lengths (A, B, C) for α -D-glucopyranose as a function of RESP-charge set

Geometry	Charge scaling factor	RESP restraint (k_{resp})	A (Å)	B (Å)	C (Å)	A (%)	B (%)	C (%)	Mean (%)	V (%)	Average potential energy (kcal mol ⁻¹)
A ^a	1.0	0.000	-	-	-	-	-	-	-	-	-
B	1.0	0.000	19.423	27.949	21.484	-4.4	-5.9	8.0	6.1	-2.4	-1057
C ^b	0.9	0.000	20.885	27.968	20.105	2.7	-5.8	1.0	3.2	-2.1	-519
A ^a	1.0	0.005	-	-	-	-	-	-	-	-	-
B	1.0	0.005	19.346	27.925	21.620	-4.8	-6.0	8.6	6.5	-2.2	-1192
A	1.0	0.010	20.588	29.072	19.604	1.3	-2.1	-1.5	1.6	-2.3	-1392
B	1.0	0.010	19.322	27.893	21.687	-4.9	-6.1	9.0	6.6	-2.0	-1408
A	0.9	0.010	20.699	29.302	19.662	1.8	-1.3	-1.2	1.4	-0.7	-787
A	1.0	0.015	20.570	29.136	19.603	0.8	-1.9	-1.5	1.4	-2.6	-1604
B	1.0	0.015	19.266	27.846	21.766	-5.4	-6.2	9.4	7.0	-2.3	-1626
A	1.0	0.020	20.547	29.144	19.630	1.1	-1.9	-1.4	1.5	-2.2	-1759
B	1.0	0.020	19.261	27.849	21.803	-5.4	-6.2	9.6	7.1	-2.1	-1782
C ^b	1.0	Mulliken ^c	18.844	27.411	21.732	-7.5	-7.7	9.2	8.1	-6.0	-2676
B	1.0	DMA ^d	19.218	27.754	21.754	-5.4	-6.6	9.3	7.1	-2.7	-1536
Experiment [18]			20.372	29.701	19.901						

^aConverts spontaneously to B-geometry.

^bHydrogen bond network disrupted, accompanied by loss of symmetry.

^cCharges computed from a HF/6-31G* wavefunction.

^dCharges (monopoles) taken from a distributed multipole analysis of the HF/6-31G* wavefunction.

Table 2Inter-oxygen ($\text{\AA}$) computed with a restraint weight of 0.01 for α -D-glucopyranose

Inter-oxygen distance	Experiment [18]	A-Geometry	B-Geometry
D1	2.78	2.69	2.80
D2	2.78	2.73	2.84
D3	3.02	2.72	2.74
D4	2.71	2.66	2.73
D5	2.78	2.76	2.78
D6	2.71	2.71	2.75
D7	3.35	3.09	3.11
D8	2.82	2.75	2.76
D9	2.85	2.73	2.78
D10	2.69	2.69	2.74
D11	2.76	2.75	2.83

Table 3Partial atomic charges (a.u.) for selected charge models computed for α -D-glucopyranose

Atom	$k_{\text{rstr}} = 0.00$	$k_{\text{rstr}} = 0.01$	Mulliken	DMA
C1	0.3942	0.2252	0.4171	0.5434
C2	0.2882	0.2474	0.1908	0.2178
C3	0.4057	0.1484	0.1117	0.2553
C4	-0.0184	0.0492	0.1113	0.2749
C5	0.3994	0.2057	0.1383	0.1476
C6	0.3338	0.2543	0.0105	0.2059
O1	-0.7528	-0.6887	-0.7391	-0.7756
O2	-0.7010	-0.6640	-0.7655	-0.7204
O3	-0.7471	-0.6732	-0.7672	-0.7482
O4	-0.7042	-0.6607	-0.7660	-0.7078
O5	-0.6195	-0.4966	-0.6787	-0.5303
O6	-0.7218	-0.6805	-0.7427	-0.6682
HO1	0.4867	0.4772	0.4563	0.4413
HO2	0.4252	0.4281	0.4520	0.4371
HO3	0.4462	0.4357	0.4522	0.4414
HO4	0.4570	0.4340	0.4482	0.4427
HO6	0.4393	0.4266	0.4426	0.4272
H1	0.0707	0.1186	0.1869	0.0544
H2	0.0526	0.0978	0.1903	0.0603
H3	0.0306	0.1188	0.1773	0.0363
H4	0.0453	0.0754	0.1691	0.0219
H5	0.0648	0.1295	0.2036	0.0503
H61	-0.0446	-0.0080	0.1556	0.0761
H62	-0.0303	0.0003	0.1451	0.0171