Conformational Sensitivity of Polyether Macrocycles to Electrostatic Potential: Partial Atomic Charges, Molecular Mechanics, and Conformational Prediction

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Abstract. Molecular recognition (whether by enzymes, the immune system, or chelating ligands) depends critically on molecular conformation. Molecular mechanics predicts energetically favorable molecular conformations by locating low energy conformations using an empirical fit of molecular potential energy as a function of internal coordinates. Molecular mechanics analysis of 18-crown-6 demonstrates that the nonbonded term (primarily the electrostatic part) is the largest contributor to the conformational energy. Nevertheless, common methods of treating the electrostatic interaction for 18-crown-6 yield inconsistent values for conformational energies partly because partial charges assigned to each atom can change with conformation due to through-space inductive effects which are not considered in most molecular mechanics programs. Similar findings from several other groups are reviewed to support our conclusions. We argue for care and caution in predicting conformational preferences of molecules with two or more highly polar atoms. We also discuss the desirability of using an empirical method of partial charge determination such as the charge equilibration algorithm of Rappé and Goddard (or a suitable generalization which includes polarization) as a method of including these effects in molecular mechanics and molecular dynamics calculations.

Key words: 18-Crown-6, conformations, molecular mechanics, electrostatics, charges.

1. Introduction

Since their discovery in 1967 by Pedersen, cyclic polyethers such as crown ethers have served as a prototypical model of molecular recognition [1]. The selectivity of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6) for K^+ ion over Na^+, Rb^+, and Li^+ ions in solution is remarkable since these ions are difficult to separate by most chemical means [2]. One explanation of this selectivity is the cavity-size theory which argues that Rb^+ and Cs^+ ions are too big to fit into the cavity of 18-crown-6 and that Na^+ and Li^+ are too small to form attractive interactions with all six polar oxygen atoms without significant distortion of the ring [3]. X-ray structures of the complexes bear out this explanation showing K^+ in sixfold coordination in a D_{3d} structure, whereas Rb^+ and Cs^+ rest atop the ring, and Na^+ has the ring curling around it [4]. The cavity-size explanation, however, has not

been supported by some experiments [5–7], implying that additional factors may be important. A large number of substituted crown ethers, lariat ethers, spherands and cryptands have been synthesized and characterized for their capability to selectively bind and separate various metal (and other) ions and neutral molecules [8,9]. Even so, because crown ethers are very flexible, with a large number of energetically accessible conformations (for example, adopting a different conformation in complexes than when free), they constitute a difficult challenge for theoretical treatment. A fundamental understanding of the molecular mechanism of selectivity is still incomplete [7,10].

Many computational methods have been successfully employed to investigate the structure of crown ethers and their binding to metal ions [11–23]. The first attempt was a CNDO semiempirical calculation by Yamabe *et al.* [11]. Although the CNDO method has been superseded by modern techniques, the qualitative conclusions of this work are informative and still believed to be correct: that selectivity is controlled by differential solvation. Later calculations by others clarified a number of issues, for example the role of structural flexibility and cyclic preorganization [14]. The most recent *ab initio* calculations by Glendening, Johnson and Feller [18] are state-of-the-art, reasonably accurate *ab initio* SCF calculations and even include the effect of solvation to the extent of two water molecules. Other recent work has also used sophisticated *ab initio* SCF calculations with correlation [19] or density functional theory [20,21]. Much of the previous work has investigated the relative energies of crown ether conformations, seeking to fit the parameters which are used in molecular mechanics force fields. Additional work has empirically simulated solution phase behavior directly [22–26].

Many recent examinations of metal ion/18-crown-6 complexes have been performed by molecular mechanics methods [12,14,17,27–32]. Molecular mechanics is a well-developed and very reliable method of structure determination for hydrocarbon systems. As will be shown in this paper, however, the conformational energy of polyethers is controlled by the electrostatic interactions between polar oxygen atoms, (and between oxygen atoms and other atoms) and these are less reliably parameterized. The MM2 force field of Allinger and coworkers [33] uses bond dipoles to represent these interactions. Other force fields, like AMBER [34], use partial charges on each atom (or possibly also in lone pairs). In addition to differences in the form of the electrostatic interaction, there is no general consensus on how to determine the partial atomic charges when used [35].

Because it can be hundreds of times faster than full-scale *ab initio* calculations, molecular mechanics has enormous promise in unraveling the conformational preferences and binding specificity of molecular recognition agents. Since there are so many possible variants of molecular recognition candidates, each with multiple low-energy conformations, it is not possible to do highly accurate quantum chemical calculations on each ligand candidate. The assumption of the molecular mechanics method is that the interactions between atoms in molecules are alike from one molecule to another. Bond lengths, and bond angles of similar

types of atoms vary only slightly from one molecule to another. Force constants for stretching and bending have also been found to be transferable for similar atoms. Torsion barriers and nonbonded parameters (van der Waals repulsions, etc.) are also parameterized. Because molecular mechanics calculations are inherently much simpler and faster than *ab initio* quantum chemical calculations, density functional calculations, or even semiempirical calculations, a reliable, easy-to-use molecular mechanics program that can predict the low-energy conformations of a wide variety of molecules is needed. There are currently dozens of 'force fields' commercially available in sophisticated software products (individual researchers often modify the default parameters, leading to hundreds of variants). Chemists routinely use these programs to locate the equilibrium structures or to study the dynamical behavior of interesting molecules: drugs, enzymes, chelating agents, etc. [36].

This paper investigates and compares methods for computing the electrostatic portion of the steric energy used by a few standard, widely-available force fields. Of course, ultimately all molecular interactions are electrostatic, but in practical application the molecular mechanics method neglects all but a few forms of this interaction. The electrostatic attraction (and repulsion) of atoms bonded to each other (a 1–2 interaction) is normally expanded about the equilibrium position (harmonic, cubic, quartic, and Morse potential forms are all common). The electrostatic interaction between two atoms each bonded to a common atom (a 1–3 interaction) is also expanded about the equilibrium bond angle to give a harmonic well, or higher-order approximation if needed. The electrostatic interaction between atoms neither bonded to each other nor to a common atom is also split up into a van der Waals term (with a weak dispersion well and a steep, repulsive component due mostly to electron–electron repulsion) and a Coulomb term proportional to the charges on the two atoms and decreasing with the distance between them (the MMn force fields of Allinger [33] uses the interaction of bond dipoles rather than point charges). When structure and conformational energy differences are not in agreement with experimental data, agreement is improved by fitting the torsional potential (1–4 interactions) for rotation about dihedral angles. The torsional potential is usually assumed to be a Fourier series of not more that three terms. It should not be surprising that the torsional parameters used by a force field depend on the way the non-bonded interactions are treated [37]. The myriad variations of force field functional forms and parameterization that exist today is a sign of a field in healthy ferment, but it is also confusing and frustrating for the non-practitioner who wants quick answers to important chemical questions. This paper will illustrate a trap into which the inexperienced user of molecular mechanics may fall. In addition, we will point out an important electrostatic effect that is not included in most common force fields despite numerous previous studies showing its importance.

Several groups are presently involved in developing suitable molecular mechanics parameter sets appropriate for polyethers and metal ion complexes with polyethers [12,14,19,21,31,38–40]. One effect that is just now being incorporated is the

'gauche effect' which favors $\pm 60°$ torsion angles between 1–4 oxygens [39–43]. In spite of intense effort, much work is still needed. One approach, that of Hay and coworkers, [31] treats the interaction of a metal ion with a ligand as a chemical bond (a 1–2 interaction). For example, K^+ is considered to be covalently bound to all six oxygens in 18-crown-6 when complexed. This treatment requires stretching, bending and torsion parameters for those coordinates involving a K^+ ion. This approach may be empirically justified when sufficient data are available; however, it will not give correct dynamical behavior when solution phase simulations are done in conditions where the metal ion can exchange between binding with the ligand and solvation by the solvent.

Another approach pursued by several groups is to model the interaction of metal ion and oxygen (or other) atoms as a standard nonbonded interaction: a combination of van der Waals and electrostatic terms. The problem here is the determination of partial atomic charges. Some researchers use the charges calculated from *ab initio* calculations [18,19,27,38]. Some researchers use charges from good-quality semiempirical calculations [44–46]. Yet another method is to empirically fit the charges to reproduce computed relative energies of several conformations [19]. The new charge equilibration method of Rappé and Goddard [47] has promise because it is fast. This method, similar to some previous electronegativity equalization methods [29, 48–54], uses atomic information about ionization potentials and electron affinities to iteratively solve a set of equations which equalize the chemical potential in all atoms of the molecule subject to the constraint of total charge. The charge equilibration method has an advantage over other electronegativity equalization methods in that it takes geometry and distance into account by approximating Coulomb integrals rather than just using bonding relationships. This method has proved very illuminating in our investigations so far, but will need to be carefully calibrated by comparison with accurate data. An additional effect not yet included in most force fields is atomic polarization, but inclusion of polarization into the charge equilibration algorithm is, in principle, well-defined [27,55]. We will come back to this point again in the discussion.

It might be erroneously concluded that one of the advantages of the bond-distance/force-constant method of Hay [31] and others [7] is that the question of determining partial charges need not be faced since ion–oxygen interactions are parameterized with bond lengths and force constants. In fact, partial charges must be determined for the interaction of oxygens with other parts of the molecule or with other nearby molecules, and the electrostatic interaction between the ion and other atoms must still be treated explicitly (although Hay neglects all electrostatic interactions of the ion). The real question is whether the partial charges that would be needed, in combination with the repulsive component of the van der Waals term, to give a proper description of the bond length and force constant are also appropriate for describing the other intramolecular (and for dynamics simulations, intermolecular) electrostatic interactions. If so, then the additional data and fitting

needed to implement the bond-length/force-constant method may not be needed and could restrict the geometry of the molecule unnecessarily.

It is not uncommon for force fields to use modified Coulomb forces to simulate the effect of solvents [56]. For example, some force fields use a distance dependent dielectric constant (making the electrostatic potential fall off as r^{-2}). A dielectric constant other than unity is sometimes used. Both of these modifications weaken the effect of the electrostatic forces and may be useful in simulating molecules in solvent; however, we defer discussion of any of these modifications and concentrate our efforts in the gas phase. There are two reasons for this choice: (1) Understanding conformational preferences in the gas phase should be a prerequisite to the more difficult condensed phase problem, and (2) gas phase experimental results are now beginning to be available [57–60] for comparison purposes. Experimental gas phase studies of crown ether binding, for example, have included mass spectrometric fragmentation information [59,60], and relative binding affinities [57,58].

2. Methods and Results

As a starting place for the calculations of this paper, we began with the results of Sun and Kollman [61] in which molecular dynamics was used to search for low energy conformations of 18-crown-6. We compared the energies of the 30 lowest energy conformations of uncomplexed 18-crown-6 as determined using the AMBER force field by Sun and Kollman to the relative energies of the same conformations as determined using the DREIDING force field [62] from the program CERIUS, and the Universal Force Field (UFF) [63], also from CERIUS. We chose these three force fields because they all use partial atomic charges for the electrostatic interaction, and they were available to us. We used several different methods of determining the charges. In all cases, the major differences in energy were controlled by the electrostatic interactions, as expected. When the charge equilibration algorithm [47] or the semiempirical AM1 (and Mulliken population analysis) [64] method were used to determine the charges, the oxygen charges were quite different in the different conformations. The changes from one conformation to another were 10 to 15%. This same effect is seen in the best *ab initio* calculations, though not to the same degree [18]. Since the electrostatic interactions in fact control which conformation is of minimum energy, a 15% error in assigning the charge changes the energy ordering of conformations. Molecular mechanics computations were performed on a Silicon Graphics PI 4D/35 workstation using the program CERIUS 3.2 from Molecular Simulations, Inc. Semiempirical AM1 calculations were done using the program HYPERCHEM 3.0 from Autodesk, Inc. running on PC compatibles. Details of the calculations are described below.

The first calculations were performed using AMBER 3.0 supplemented with the parameters of Billeter *et al.*[27]. Sun and Kollman [61] reported the 30 lowest energy conformations of 18-crown-6 using this force field along with evidence that these conformations were populated with approximately Boltzmann weighting in

TABLE I. Relative energies of 30 low energy conformations of 18-crown-6 determined using five different methods. The last five rows show standard deviations for the five contributions to the steric energy. An asterisk indicates that the reference geometry did not lead to a local minimum with the same assignment of torsion angles.

Conf. #	amb/bil[a]	amb/am1[a]	amb/qeq[a]	uff/qeq[a]	dri/qeq[a]
1	0.00	0.83	1.31	*7.31	2.07
2	1.19	1.27	2.64	12.79	5.70
3	1.23	0.84	6.63	*4.97	6.32
4	1.43	*2.19	3.61	*6.40	4.86
5	1.48	1.83	6.78	8.73	6.07
6	1.56	1.60	0.63	4.66	2.71
7	1.59	1.15	4.76	13.68	6.61
8	1.59	1.51	1.93	17.22	5.71
9	1.68	1.42	4.57	9.95	6.40
10	1.71	2.52	7.81	16.27	6.89
11	1.81	0.00	0.22	6.95	2.28
12	1.87	1.52	6.90	10.37	7.08
13	1.92	0.18	2.45	5.86	3.33
14	2.02	5.01	29.96	*7.22	17.64
15	2.07	2.19	0.00	0.00	0.00
16	2.14	2.54	9.52	16.47	7.61
17	2.17	*3.53	2.32	*4.47	3.51
18	2.16	1.37	5.74	*5.12	5.39
19	2.25	1.39	6.94	*10.12	8.18
20	2.24	2.12	3.85	9.08	4.57
21	2.25	3.41	2.57	*5.32	3.40
22	2.28	1.67	2.14	*1.48	6.88
23	2.30	1.83	4.36	5.37	4.58
24	2.32	*0.87	*17.82	*7.31	11.01
25	2.34	1.99	5.37	10.53	7.10
26	2.36	2.11	5.54	8.80	6.81
27	2.48	3.12	*8.21	13.00	9.48
28	2.51	*4.04	9.33	*7.23	8.71
29	2.51	0.64	4.16	5.82	3.93
30	2.51	0.73	0.7	13.01	2.19
Bond	0.11	0.07	0.09	0.53	0.20
Angle	0.80	0.63	0.48	1.90	1.09
Torsion	1.19	1.72	1.12	1.06	0.86
vdW	0.37	0.38	0.54	0.88	0.66
Coul	1.95	2.57	5.35	3.39	4.03

[a] The notation represents: force field/charge method: amb = AMBER force field (as modified in [27]); bil = charges from [27]; am1 = charges from Mulliken population analysis of AM1 semiempirical wavefunction; qeq = charges from Qeq algorithm of [47]; uff = UFF force field of [63]; dri = DREIDING force field of [62].

Conformation 1

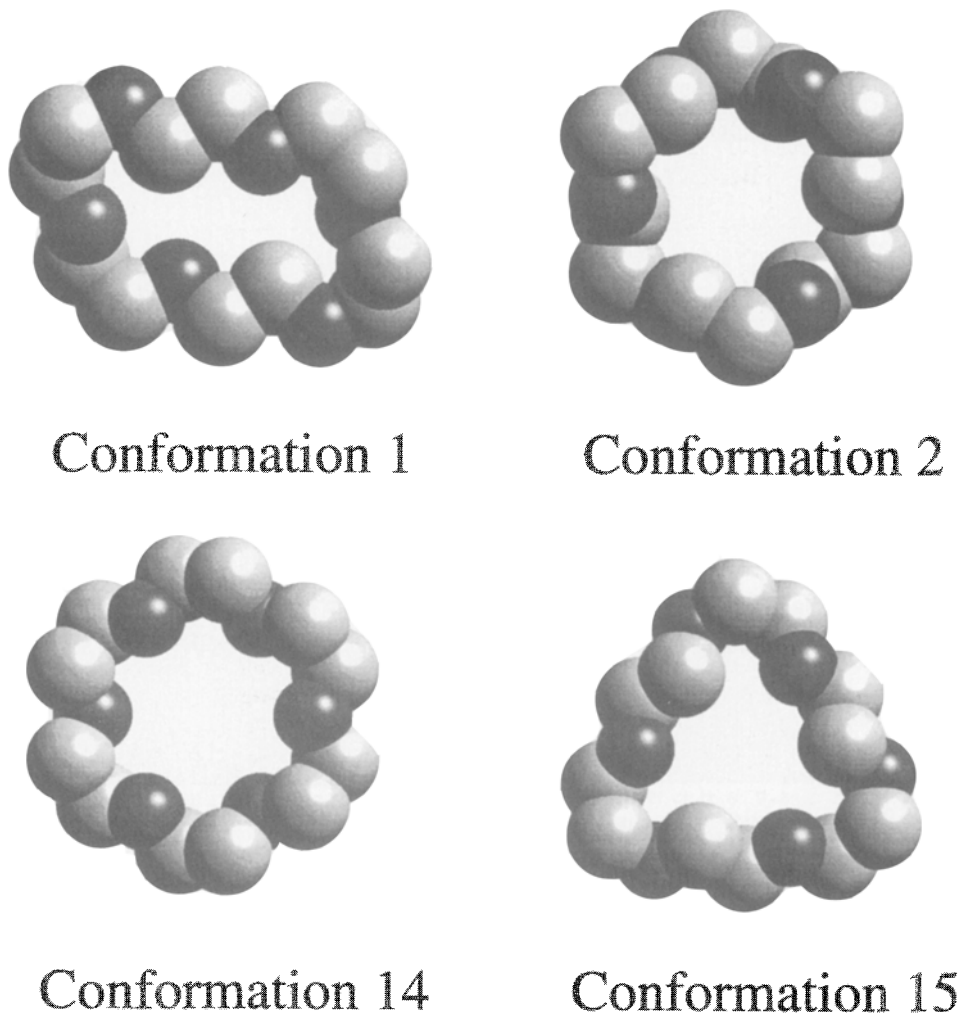## Conformation 2

Conformation 14

Conformation 15

Fig. 1.   Pictures of four important configurations of 18-crown-6. Carbon atoms are light, oxygen atoms are dark, hydrogen atoms are not shown. Numbering is energy ordering from [61]. Configuration No. 1 has C_i symmetry and is the structure of uncomplexed 18-crown-6. Configuration No. 14 has D_{3d} structure with the six oxygen atoms oriented so as to be pointing toward the center of the cavity; this is the configuration of many metal ion/18-crown-6 complexes. Configuration No. 2 also has D_{3d} symmetry but has the oxygens rotated away from the cavity. Configuration No. 15 has C_3 symmetry.

the molecular dynamics calculations (drawings of four of these 30 conformations are shown in Figure 1). The conformational energies are listed in Table I in order of energy, relative to the lowest energy conformer. The thirtieth conformation has an energy of 2.51 kcal/mol higher than the lowest. We were able to reproduce the reported energies and energy ordering acceptably. Minor disagreements with [61] may be due to the fact that the charges given in [61] are rounded to three decimal places so that, if they are all added, the molecule is not neutral. We
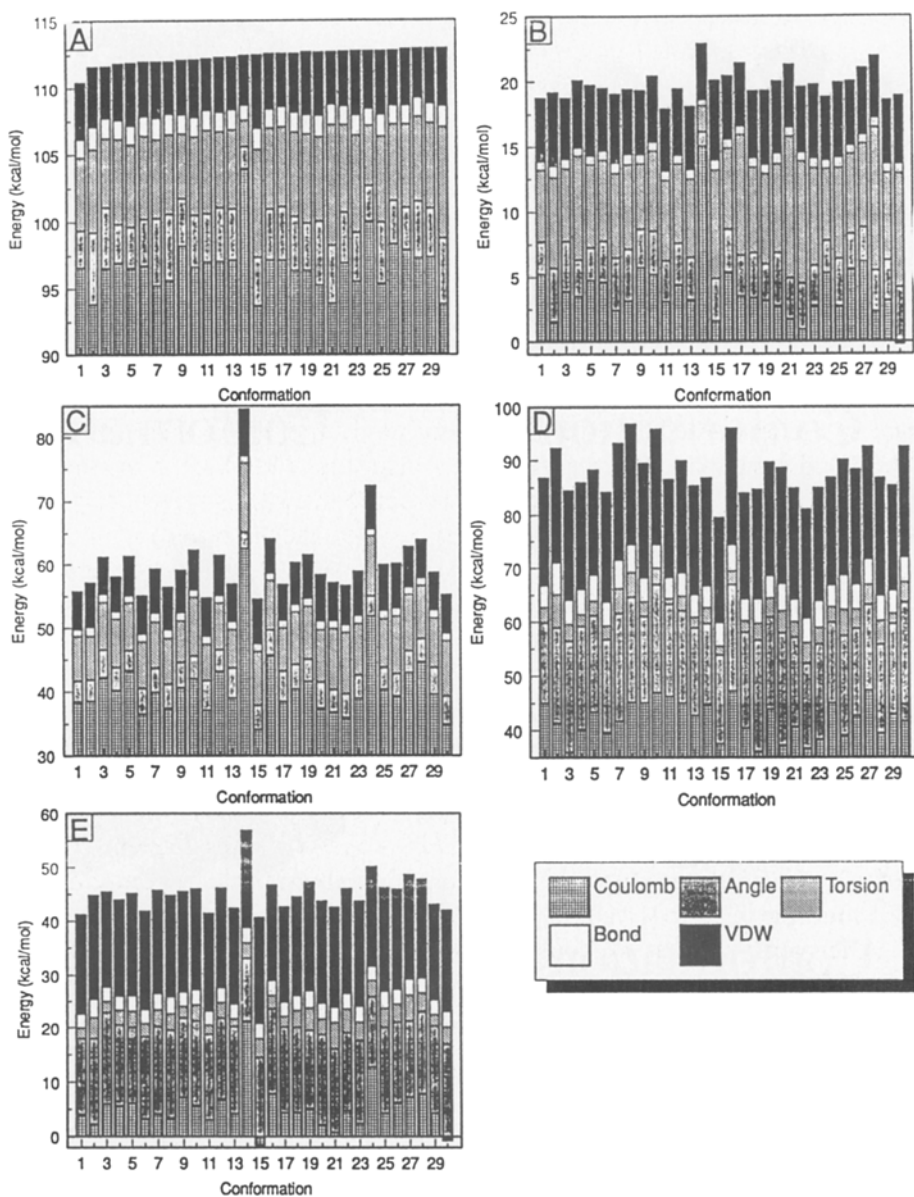
Fig. 2. Bar graphs showing individual contributions to the total steric energy of 18-crown-6 for 30 different conformations using five different force fields. Contributions are, in order from the bottom: Coulomb or electrostatic, angle bending, torsion, bond stretching, van der Waals interaction. Figure 2a is for the AMBER force field (modified) with charges as used in [61]. The 30 configurations are numbered with energy ordering with this force field. Figure 2b is for the modified AMBER force field used in [61] with charges determined from the AM1 semiempirical method and Mulliken population analysis. Figure 2c is for the AMBER force field (modified) used in [61] with charges determined using the QEq algorithm [47]. Figure 2d is for the UFF force field [63] and QEq charges. Figure 2e is for the DREIDING force field [62] and QEq charges. The important conclusion of this figure is that the ordering of the conformations is different for each force field. The major difference between the contributions is in the electrostatic contribution. Note that the vertical scale is different for each graph.

used the following charges (O: $-0.446e$, C: $+0.210e$, H: $0.0205e$, e = unit of electron charge). Table I also lists the standard deviation shown by the five different contributions to the conformational energy: bond stretching, angle bending, torsion, van der Waals, and Coulomb (or electrostatic) contributions. These contributions are shown in bar graph form in Figure 2a. It is interesting to note that for this force field, the electrostatic contribution is typically 85% of the total steric energy; however, differences are much more important than total contribution. So it is important to note that, with few exceptions, the contributions due to bond stretching, angle bending, torsion, and van der Waals interactions are roughly constant among the 30 conformations studied. The largest differences between conformations are due to different amounts of electrostatic contribution. The dispersion of the 30 electrostatic contributions as measured by the standard deviation is almost twice that of the torsion contribution, and three times that of the angle bending term. The bond stretching and van der Waals dispersions are both even smaller. We conclude that the Coulomb term dominates conformational preference. In response to electrostatic forces, the molecule tries to minimize the repulsion of the highly charged oxygen first by twisting torsional angles so oxygens are farther apart, and then by bending angles. Additional calculations below will reinforce this pattern.

We have identified conformations of 18-crown-6 using the notation of Uiterwijk *et al.* [17] and Sun and Kollman [61], assigning each of the 18 torsional angles around the ring to be + if the angle was between $0°$ and $120°$, − if the angle was between $0°$ and $-120°$, and 0 if the torsion angle was between $120°$ and $240°$. Thus a conformation is described by a sequence of 18 + signs, − signs, or 0's. In the remainder of the paper, when comparing conformations, two conformations are considered to be equivalent simply if they have the same sequence (or if they differ by a shift of a multiple of three signs, or reversal of the sequence and a shift by a multiple of three signs).

After verifying that we could reproduce the results of Sun and Kollman, we asked if these same conformations are low energy conformations using different force fields. It is not expected that the precise geometry of a local minimum from one force field will be a local minimum for another force field, or that the relative energies at precisely the same geometry should be compared. Instead, we compare local minima for different force fields to see that their torsion angles are within the same range (either +, −, or 0), and we compare the steric energies relative to a common low energy conformation.

A second set of minimizations was done using the AMBER 3.0 force field as modified by Billeter *et al.* [27], but using charges calculated by the AM1 semiempirical method using Mulliken population analysis [64] (implemented in HYPERCHEM 3.0). The AM1 method was chosen among the many semiempirical electronic structure methods because it is felt by some to be more reliable [19,46]. Although Mulliken population is well-known to be unreliable for calculating charges [65], the charges show the same important effect as other methods. Starting at the reference geometry with torsion angles at $60°(+)$, $-60°(-)$, or

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TABLE II. Summary of partial atomic charges for 18-crown-6 using AMBER force field and AM1 Mulliken charges.

C#	C_{avg}	C_{min}	C_{max}	H_{avg}	H_{min}	H_{max}	O1	O4	O7	O10	O13	O16
1	−0.020	−0.051	−0.006	0.081	0.066	0.107	−0.280	−0.279	−0.288	−0.280	−0.279	−0.288
2	−0.034	−0.052	−0.015	0.090	0.074	0.108	−0.293	−0.293	−0.293	−0.293	−0.293	−0.293
3	−0.026	−0.050	−0.011	0.082	0.065	0.107	−0.276	−0.277	−0.283	−0.276	−0.277	−0.283
4	−0.027	−0.052	−0.007	0.084	0.063	0.112	−0.270	−0.285	−0.286	−0.270	−0.285	−0.286
5	−0.024	−0.052	−0.007	0.082	0.065	0.115	−0.290	−0.287	−0.267	−0.267	−0.279	−0.287
6	−0.023	−0.051	−0.006	0.082	0.067	0.119	−0.277	−0.285	−0.273	−0.280	−0.289	−0.280
7	−0.030	−0.056	−0.013	0.086	0.067	0.108	−0.283	−0.278	−0.293	−0.289	−0.291	−0.277
8	−0.030	−0.059	−0.011	0.086	0.065	0.112	−0.290	−0.289	−0.282	−0.280	−0.277	−0.287
9	−0.024	−0.054	−0.006	0.081	0.061	0.113	−0.281	−0.270	−0.279	−0.278	−0.270	−0.289
10	−0.022	−0.053	−0.005	0.081	0.064	0.107	−0.271	−0.276	−0.277	−0.285	−0.291	−0.277
11	−0.027	−0.056	−0.006	0.083	0.064	0.118	−0.272	−0.290	−0.270	−0.272	−0.290	−0.270
12	−0.027	−0.056	−0.008	0.084	0.065	0.115	−0.288	−0.280	−0.292	−0.276	−0.268	−0.281
13	−0.027	−0.055	−0.006	0.083	0.063	0.117	−0.272	−0.290	−0.266	−0.274	−0.289	−0.270
14	−0.011	−0.011	−0.011	0.071	0.061	0.080	−0.260	−0.260	−0.260	−0.260	−0.260	−0.260
15	−0.024	−0.045	−0.009	0.084	0.070	0.108	−0.285	−0.292	−0.285	−0.292	−0.285	−0.292
16	−0.023	−0.053	−0.006	0.081	0.065	0.108	−0.288	−0.290	−0.276	−0.262	−0.272	−0.278
17	−0.027	−0.055	−0.008	0.084	0.065	0.117	−0.289	−0.275	−0.277	−0.282	−0.279	−0.280
18	−0.027	−0.052	−0.007	0.083	0.064	0.113	−0.285	−0.288	−0.270	−0.283	−0.275	−0.279
19	−0.028	−0.059	−0.009	0.084	0.066	0.119	−0.279	−0.273	−0.283	−0.285	−0.278	−0.283
20	−0.026	−0.052	−0.007	0.084	0.064	0.108	−0.286	−0.280	−0.273	−0.292	−0.277	−0.294
21	−0.030	−0.055	−0.011	0.087	0.067	0.108	−0.282	−0.283	−0.292	−0.288	−0.288	−0.288
22	−0.031	−0.057	−0.009	0.087	0.065	0.112	−0.285	−0.279	−0.290	−0.288	−0.281	−0.287
23	−0.027	−0.058	−0.008	0.085	0.067	0.115	−0.282	−0.281	−0.275	−0.284	−0.286	−0.293
24	−0.020	−0.051	−0.006	0.081	0.066	0.108	−0.279	−0.280	−0.288	−0.279	−0.280	−0.288
25	−0.031	−0.057	−0.008	0.087	0.069	0.114	−0.282	−0.286	−0.282	−0.275	−0.294	−0.290
26	−0.023	−0.050	−0.006	0.081	0.062	0.114	−0.271	−0.278	−0.278	−0.269	−0.288	−0.281
27	−0.024	−0.052	−0.009	0.082	0.062	0.112	−0.283	−0.271	−0.269	−0.290	−0.279	−0.277
28	−0.033	−0.059	−0.012	0.087	0.065	0.117	−0.291	−0.283	−0.277	−0.279	−0.277	−0.279
29	−0.027	−0.052	−0.006	0.083	0.065	0.115	−0.266	−0.289	−0.274	−0.266	−0.289	−0.274
30	−0.030	−0.054	−0.009	0.086	0.067	0.113	−0.283	−0.295	−0.286	−0.277	−0.276	−0.295
Max	−0.011	−0.011	−0.005	0.090	0.074	0.119	−0.260	−0.260	−0.260	−0.260	−0.260	−0.260
Min	−0.034	−0.059	−0.015	0.071	0.061	0.080	−0.293	−0.295	−0.293	−0.293	−0.294	−0.295

180°(0), we performed standard molecular mechanics energy minimizations of the 30 conformations in HYPERCHEM using the same force field parameters as before but different partial atomic charges. The ordering of the conformations is very different from the previous ordering (Figure 2b and Table I). Conformation No. 11 is the lowest in this calculation with No. 13 close in energy. Conformation No. 14 has the highest energy of these 30 conformations, 5.01 kcal/mole higher than the lowest configuration. Again, the standard deviation of the electrostatic contributions to

TABLE III. Summary of partial atomic charges for 18-crown-6 using AMBER force field of Billeter, *et al.* [27] with charges from the QEq algorithm [47].

C#	C_{avg}	C_{min}	C_{max}	H_{avg}	H_{min}	H_{max}	O1	O4	O7	O10	O13	O16
1	0.035	0.004	0.082	0.129	0.069	0.225	−0.574	−0.589	−0.600	−0.574	−0.589	−0.600
2	0.027	0.012	0.041	0.135	0.104	0.180	−0.597	−0.597	−0.597	−0.597	−0.597	−0.597
3	0.038	−0.001	0.084	0.127	0.070	0.195	−0.579	−0.593	−0.585	−0.579	−0.593	−0.585
4	0.037	−0.004	0.094	0.127	0.064	0.202	−0.578	−0.568	−0.604	−0.578	−0.568	−0.604
5	0.037	−0.002	0.068	0.126	0.054	0.208	−0.615	−0.594	−0.569	−0.533	−0.567	−0.601
6	0.034	0.001	0.092	0.129	0.070	0.229	−0.588	−0.596	−0.571	−0.598	−0.601	−0.572
7	0.032	0.002	0.088	0.131	0.063	0.195	−0.586	−0.562	−0.603	−0.590	−0.611	−0.588
8	0.032	−0.002	0.059	0.131	0.064	0.220	−0.602	−0.591	−0.573	−0.589	−0.589	−0.600
9	0.038	0.005	0.100	0.124	0.068	0.241	−0.572	−0.556	−0.568	−0.571	−0.581	−0.601
10	0.038	−0.004	0.086	0.127	0.064	0.194	−0.551	−0.585	−0.591	−0.617	−0.602	−0.576
11	0.035	0.002	0.081	0.128	0.062	0.231	−0.563	−0.602	−0.579	−0.563	−0.602	−0.579
12	0.035	0.002	0.082	0.126	0.057	0.215	−0.598	−0.607	−0.600	−0.558	−0.539	−0.568
13	0.035	0.002	0.081	0.127	0.061	0.233	−0.564	−0.603	−0.570	−0.563	−0.602	−0.579
14	0.058	0.058	0.058	0.106	0.059	0.153	−0.542	−0.542	−0.542	−0.542	−0.542	−0.542
15	0.029	0.006	0.064	0.136	0.090	0.194	−0.593	−0.617	−0.593	−0.617	−0.593	−0.617
16	0.040	−0.003	0.081	0.123	0.048	0.210	−0.614	−0.605	−0.559	−0.521	−0.565	−0.594
17	0.037	−0.002	0.092	0.127	0.067	0.236	−0.603	−0.593	−0.556	−0.590	−0.585	−0.570
18	0.035	0.001	0.085	0.129	0.072	0.219	−0.583	−0.596	−0.584	−0.589	−0.592	−0.586
19	0.032	0.002	0.079	0.129	0.065	0.201	−0.563	−0.556	−0.574	−0.595	−0.607	−0.612
20	0.033	−0.004	0.082	0.132	0.056	0.200	−0.593	−0.582	−0.561	−0.617	−0.588	−0.626
21	0.031	0.002	0.076	0.135	0.087	0.194	−0.613	−0.590	−0.615	−0.591	−0.594	−0.602
22	0.028	0.000	0.044	0.135	0.076	0.206	−0.603	−0.593	−0.598	−0.597	−0.610	−0.591
23	0.032	0.000	0.087	0.131	0.057	0.205	−0.588	−0.573	−0.557	−0.615	−0.591	−0.620
24	0.048	0.004	0.084	0.117	0.059	0.219	−0.565	−0.534	−0.543	−0.558	−0.585	−0.602
25	0.031	0.003	0.086	0.131	0.056	0.186	−0.609	−0.589	−0.576	−0.555	−0.602	−0.594
26	0.037	0.004	0.102	0.126	0.061	0.240	−0.563	−0.572	−0.579	−0.590	−0.600	−0.575
27	0.037	−0.008	0.084	0.127	0.058	0.183	−0.569	−0.538	−0.577	−0.596	−0.612	−0.601
28	0.038	−0.003	0.079	0.125	0.052	0.220	−0.597	−0.605	−0.594	−0.576	−0.530	−0.558
29	0.035	0.005	0.082	0.127	0.060	0.218	−0.572	−0.602	−0.567	−0.572	−0.602	−0.567
30	0.029	−0.004	0.084	0.136	0.083	0.176	−0.594	−0.607	−0.596	−0.615	−0.590	−0.628
Max	0.058	0.058	0.102	0.136	0.104	0.241	−0.542	−0.534	−0.542	−0.521	−0.530	−0.542
Min	0.027	−0.008	0.041	0.106	0.048	0.153	−0.615	−0.617	−0.615	−0.617	−0.612	−0.628

the steric energy are considerably larger than for the other contributions, showing that electrostatic interactions are the largest determiner of conformational energy. It is useful to note that atomic point charges calculated by Mulliken population analysis of the AM1 wavefunction are generally smaller than those used by Billeter *et al.* [27]; but it is even more important to note that the charges calculated by the AM1 method are different for each conformation. These charges were determined iteratively by doing an AM1 calculation at the beginning, reference geometry for

the desired configuration, then using the force field to minimize the energy. At that configuration, another AM1 calculation was performed and the energy re-minimized using the force field. The procedure was repeated until the energy was constant to within 0.001 kcal/mol between iterations and the charges were invariant to within 0.001e. Three iterations were usually sufficient. For six conformations, the minimum energy conformation achieved did not have the same torsion angle assignments as the initial reference conformation. These are indicated in Table I with an asterisk. Table II lists the ranges of the AM1 charges on hydrogen and carbons and the individual charges on the oxygens for the 30 conformations. The differences between conformations suggests a definite intramolecular, through-space inductive effect. The lowest oxygen charges (in absolute value) are found on Conformation No. 14, the D_{3d} structure. In this conformation, the six oxygen atoms are turned into the center of the cavity (see Figure 1). In this arrangement, they would interact strongly with a positive ion, but in the free ligand, they experience the largest repulsion between the negative partial atomic charges. In this arrange-ment, the electron density that would otherwise be on the oxygen atoms leaks out to adjacent atoms on the outside of the ring to minimize the repulsive interaction. Additional minimization of the energy can take place by bond lengthening and by bending and torsional displacements to make up for the high electrostatic repul-sions between oxygen atoms, but the repulsive interactions between oxygens is clearly the reason Conformation No. 14 is the highest energy conformation in this calculation. The through-space inductive effect is also seen in the hydrogen atom charges for Conformation No. 14 which, on the average, are the lowest of any of the 30 conformations. The through-space inductive effect is also seen in the variation in charges for conformations of lower symmetry. Those oxygen atoms which point inward into the cavity always have the lowest charge (in absolute value).

A third set of minimizations was performed using the AMBER force field as supplemented by Billeter et al. [27] with atomic point charges as determined using the charge equilibration algorithm (QEq) of Rappé and Goddard [47]. The charges determined by this method tend to be larger than the ones calculated using either AM1 method or the charges used by Billeter et al. [27]. These charges were determined iteratively as described in the preceding paragraph for the AM1 Mulliken charges. In comparison, the QEq charge calculation is much faster than the AM1 calculation. In this example, Conformation No. 15 is the lowest in energy. The energy ordering of the 30 conformations is much different than for the first two methods. The highest energy conformation among these 30 is Conformation No. 14, 30 kcal/mol higher than the Conformation No. 15. For this set of calculations, two of the final local minima did not have the same torsion angle assignments as the initial reference conformations. Partial atomic charges for the 30 conformations are summarized in Table III. The charges are different from one conformation to another as in the AM1 method. For example, for Conformation No. 14, the D_{3d} structure, the charges on oxygen atoms are again smaller in absolute value than in other conformations. The same comments made regarding the variation

of partial atomic charges among conformations for AM1 calculation discussed in the previous paragraph apply to the QEq calculation. Because the QEq charges are larger than the AM1 charges or those used by [61], the magnitude of the electrostatic interaction is larger in this calculation. Approximately 50 percent of the steric energy for a conformation is the electrostatic term (Figure 2c). This alone is not significant because we are only interested in the difference in energy among conformations. In this regard, the dispersion in the electrostatic term is four times greater than the dispersion in the other contributions (Table I). As above, the electrostatic term is the largest factor in determining ordering of conformations. The oxygen charge ranges from $-0.52e$ to $-0.63e$. The constant value of $-0.466e$ chosen by Kollman is not too far out of this range, but we believe the variation of charges between conformations and between different inequivalent atoms in a single conformation is extremely important and represents a real physical effect since the same variations appear using two different methods as well as in *ab initio* calculations [18]. This point will be discussed below.

A fourth set of minimizations was performed using the universal force field (UFF)[63]. This force field is also implemented with the charge equilibration algorithm in the program CERIUS. The energy ordering of the thirty conformations is different than in the previous calculations, this time with Conformation No. 15 the lowest (Table I and Figure 2d). Conformation No. 8 is the highest of these conformations, 17.22 kcal/mol above Conformation No. 15. The standard deviation of the electrostatic contribution (Table I) again shows that electrostatic interactions are the largest determiner of relative conformational energy, although the angle bending term shows a significantly larger variation in this computation than in the previous three. It should be noted, however, that in this calculation eleven of the final local minima did not have the same torsion angle assignments as the initial reference conformation. The variation of charges between the conformations (not included here) is similar to that shown in Table III. Oxygen charges ranged from $(-0.625e$ to $-0.524e$; hydrogen charges ranged from $0.033e$ to $0.238e$; carbon charges ranged from $0.001e$ to $0.150e$). This calculation shows that partial atomic charges vary between conformations and between inequivalent atoms in the same configuration independent of the choice of force field.

A fifth set of minimizations was performed using another commercially available force field, DREIDING, using the QEq algorithm to calculate the charges. The DREIDING force field [62] is a simplified force field that uses a limited set of parameters and atom types including, for example, only one stretching force constant for all single bonds. The DREIDING force field gave a fifth different ordering of the same 30 configurations (Table I). Figure 2e graphically displays the results broken down by contributing terms. The lowest energy conformation is No. 15. The highest energy conformation (No. 14) is 17.64 kcal/mol higher than No. 15. In this calculation, all of the final local minima have the same torsion angle assignments as the reference conformation. In fact, the root-mean-square difference from the torsion angles in the AMBER constant charge calculation was

only 4°. Standard deviations of the steric energy contributions (Table I), however, show that the electrostatic term is again the most important determiner of relative conformational energy. The variation of charges between the conformations (not included here) is similar to that shown in Table III. Oxygen charges ranged from $-0.614e$ to $-0.502e$; hydrogen charges ranged from $0.044e$ to $0.234e$; carbon charges ranged from $-0.005e$ to $0.101e$. The charges determined in this calculation show the same conformational variation as the AM1 calculations and the other QEq calculations.

It is not unexpected that the calculations described above gave different results. The degree of difference is perhaps surprising, but the purpose of this paper is not to discuss the advantages or disadvantages of the several methods. Our purpose is rather to point out the sensitivity of relative conformational energies on the atomic partial charges and the method by which they are calculated. Ultimately, we seek a method of predicting conformational energy that is general (that can be used for a wide variety of molecules without recalibration) and computationally fast. Clearly, the differences shown in the above calculations cast doubt on the reliability of conformational relative energies using off-the-shelf molecular mechanics programs arbitrarily paired with an available method of determining atomic partial charges. On the other hand, one must *not* stop at this point to argue about which of the methods gave the correct answer because there is no experimental information available about either the gas phase relative energies of 18-crown-6 conformations, or even the identity of the lowest energy configuration. We think that the most important conclusion to be drawn from this evidence is that atomic partial charges must be very carefully considered for molecules such as 18-crown-6 in which there are several polar groups such as ether oxygens.

A minor comment regarding the charges calculated by the QEq algorithm is in order. Wipff *et al.* [14] point out that a partial atomic charge on oxygen of $-0.6e$ gives a dipole moment for each CH_2-O-CH_2 fragment of 18-crown-6 that is considerably in excess of the experimental dipole moment of dimethyl ether (2.48 D vs. 1.3 D). They settled on an oxygen partial atomic charge of $-0.3e$ in order to match these two dipole moments. The QEq algorithm consistently gives charges in the range of $-0.6e$. The dipole moments of individual CH_2-O-CH_2 fragments in our calculations computed with the QEq algorithms ranged from 2.2 D to 4.1 D. Fragment dipole moments from AM1 charges ranged from 0.77 D to 2.16 D while those using the constant Billeter *et al.* [27] charges ranged from 1.26 D to 1.52 D (the range is due to bond and torsion angle changes). Even though it has been argued that molecular dipole moments should be simulated with higher than experimental gas phase values to partially take polarizability into account [66], or to balance the intermolecular electrostatic interactions with a particular solvent model [67,68], it seems clear that the present parameterization of the QEq algorithm overestimates the electronegativity difference between O and C in 18-crown-6 leading to partial charges on the oxygen atoms which are too large (in absolute value).

3. Discussion

The sensitivity of relative conformational energy to changes in the electrostatic interaction has been understood for over a decade [14]. We attempt here to develop a method to quantitatively determine the accuracy needed in partial charge determination for reliable conformational prediction. The relative population of two conformations with a relative energy ΔE is given by the Boltzmann factor:

$$\frac{P_i}{P_j} = \frac{g_i}{g_j} e^{-\Delta E/kT}, \tag{1}$$

where P_i is the probability of conformation i, g_i is the degeneracy of the conformation, k is Boltzmann's constant, and T is the absolute temperature. At room temperature, and for similar degeneracies, an error in the relative energy of 1.0 kcal/mol is sufficient to make the populations in error by a factor of 5.3. Thus, the maximum error that we can allow if we expect to make predictions of conformational preference must be less than 1.0 kcal/mol. For some classes of molecules, the parameterization of molecular mechanics force fields is sufficiently mature that one can expect an accuracy of 1 kcal/mol in relative conformational energy, but considerable work remains before this accuracy is obtained for crown ethers and other molecules with multiple polar atoms. Moreover, the electrostatic modeling problem increases for larger molecules since for N atoms the number of electrostatic interactions goes as N^2 and the typical distance between atoms only increases as $N^{1/3}$.

We now seek to estimate the partial atomic charge accuracy required to give steric energies to within 1.0 kcal/mol energy. As a quick but instructive exercise, we first demonstrate a naive way to do this. The Coulomb interaction among a set of charges is given by:

$$\sum_{i<j} \frac{C_0 q_i q_j}{\epsilon R_{ij}}, \tag{2}$$

where C_0 is a constant depending on the choice of units, ϵ is the dielectric constant (1.0 for gas phase), q_i is the charge on atom i, and R_{ij} is the distance between atom i and atom j. Using equation (2), we can estimate the maximum deviation that we can tolerate for the charge on oxygen in 18-crown-6:

$$\sum_{i<j} \left(\frac{C_0 q_i (q_j + \delta)}{R_{ij}} - \frac{C_0(q_i q_j)}{R_{ij}} \right) \lesssim 1 \frac{\text{kcal}}{\text{mol}}. \tag{3}$$

Using a typical distance ($R = 4$ Å) for all distances, and a typical charge for oxygen about -0.5e, and neglecting the carbon and hydrogen atoms whose charges are smaller, gives $\delta = 0.024$e. Since each oxygen interacts with 5 other oxygens in 18-crown-6, we must divide this result by five, giving $\delta = 0.005$e. In other words,
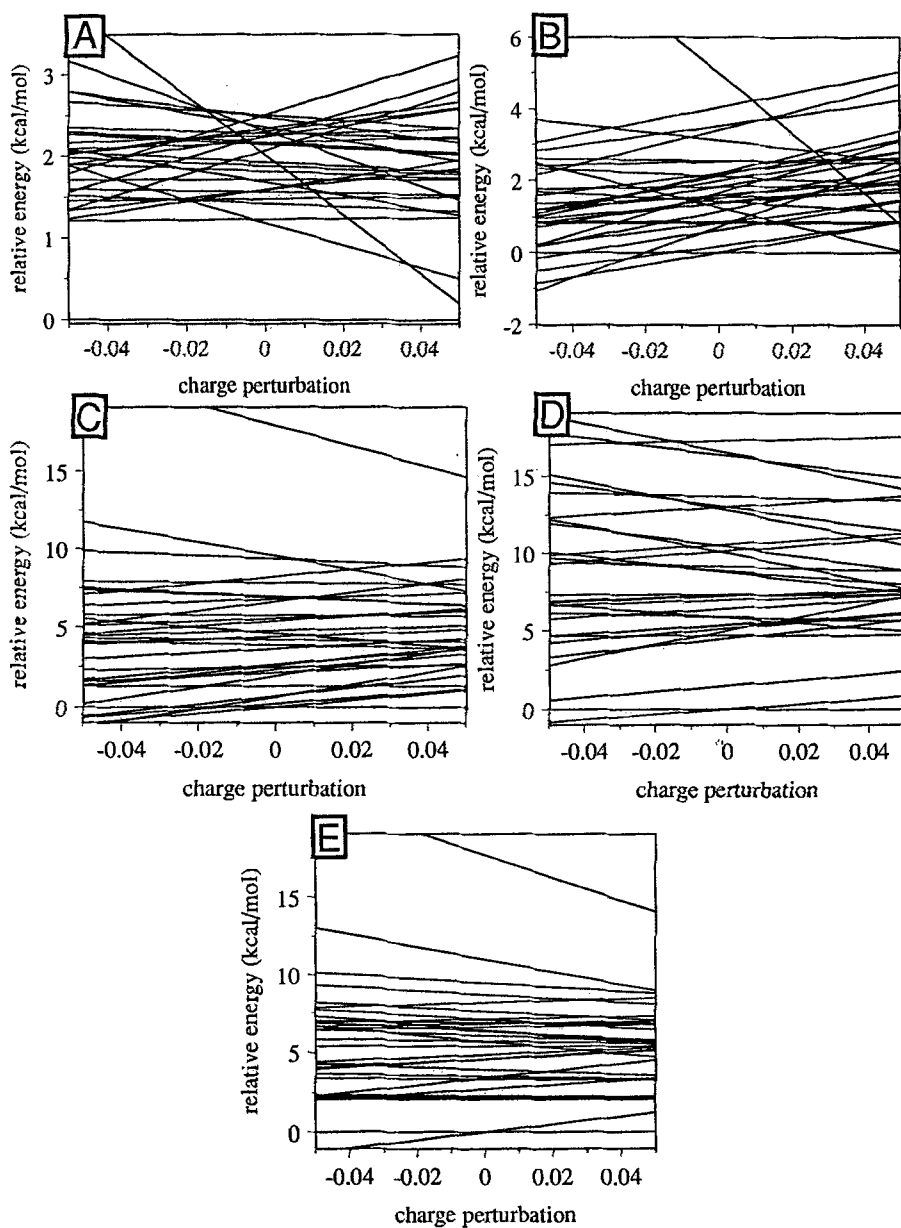
Fig. 3.   Relative conformational energies for the same five calculations in Figure 2 as a function of oxygen charge. All energies are relative to the lowest energy configuration for that force field. Oxygen charges were perturbed from the values determined by the various methods (constant charges for a, AM1 Mulliken charges for b, QEq charges for c,d, and e) with carbon and hydrogen charge perturbed proportional to the charge on the atom. Note the extreme sensitivity of conformational energy to charge. Note that the plots have different vertical scales.

the charge on each oxygen must be determined within 0.005e in order for the conformational energy to be accurate to within 1.0 kcal/mol. This estimate is considerably too conservative for reasons to be discussed next, but this simple calculation illustrates the strength of the electrostatic interaction in molecules with highly polar groups as well as the sensitivity of conformational energetics to the parameterization of the partial atomic charges. If this estimate were accurate and the effects were linear, differences in partial charges of 0.3e would result in energy changes of 60 kcal/mol. Obviously, energy differences in our calculations are smaller than this.

To quantitatively test the sensitivity of molecular mechanical conformational energies to changes in partial atomic charges in a more physically reasonable way, we tested several possible methods of perturbing the partial charges. The method described in Equation (3) is unsatisfactory because it changes the overall charge on the molecule. A more satisfactory perturbation might be to change the charge on one or more oxygen atoms with a corresponding change on one or more other oxygen atoms such that the total charge on the molecule is unchanged. Preliminary results with several such possible changes showed little effect. We did note, however, that the steric energy was strongly dependent on the charge *difference* between oxygen and carbon. Figure 3 shows the result of a calculation in which, starting from the 30 conformations described above for each of the five calculations, each oxygen atom's partial charge was changed by +0.005. Then the charges on the two carbon atoms bonded to that oxygen and the four hydrogen atoms bonded to those two carbons were changed by an amount proportional to their charges in the starting configuration but in a way such that the charge on the CH_2OCH_2 unit remained constant. After these new perturbed charges were obtained, the molecular mechanics method was used (using the force field that gave the starting configuration) to reminimize the energy to give an energy appropriate for the perturbed charges. The difference between this energy and the energy of the starting configuration with unperturbed charges was divided by 0.005e to give the derivative of configuration energy with respect to the oxygen charge. This parameter is a good numerical measure of the sensitivity of the molecular mechanics method to atomic partial charges. Assuming that a linear approximation is good, Figure 3 shows how the conformational ordering will change as the partial atomic charges are proportionally changed. For each plot, zero abscissa gives the same energies listed in Table I. The slope of each line represents the sensitivity to charge perturbation of that configuration relative to the sensitivity of the lowest energy configuration (note, however, that configuration No. 14 is off-scale in Figure 3c). It is clear from this figure that, even holding all other force field parameters constant, partial atomic charges must be determined to within 0.05e in order to have reliable predictions of the configurational populations of 18-crown-6. The back-of-the-envelope calculation of Equation (3) is superseded by this more quantitative estimate of the sensitivity of conformational energy to charge perturbations. It is instructive that the force field used by Sun and Kollman

[61] is the least sensitive to charge perturbation (note the difference in vertical scale) even though the AM1 charges are smaller in magnitude. We believe this is true because the other charge determination methods were not explicitly considered in optimizing the force fields we used in these sample calculations. We emphasize here that force field parameters and partial atomic charges are inherently related. It is important to optimize other force field parameters for those specific electrostatic interaction parameters with which they will be used. Thus, it is inadvisable to use partial atomic charges calculated by a new method without reoptimizing the entire force field. Even so, we feel that presently available force fields neglect an important electrostatic effect in that partial atomic charges are sufficiently conformationally dependent to question the validity of molecular mechanics predictions on some classes of compounds.

The most relevant calculation that reports conformational variation of atomic charge is the recent *ab initio* calculation of 18-crown-6 and its complexes by Glendening *et al.* [18]. Using natural population analysis [65], they found that the C_i conformation (Configuration No. 1) and the D_{3d} conformation (Configuration No. 14) have average oxygen atom charges which differ by 0.016e. The oxygen atoms in the D_{3d} conformation have lower charges (in absolute value) as was obtained from our calculations and is expected based on the concept of through-space induction. Other researchers have noticed similar variation of atomic charges with conformation in other molecules. Breneman and Wiberg [69] found significant partial atomic charge variation upon rotation about the C—N bond in formamide. Williams [70] calculated partial atomic charges for alanyl dipeptide from *ab initio* calculations for twelve different conformations. He found that the charges that best fit the electrostatic potential varied a great deal among the conformations. Bellido and Rullmann noted similar conformational dependence [71]. Cieplak and Kollman [68], based on previous work by Jorgensen and Gao [72], found that the relative solvation free energies of *cis* and *trans-N*-methylacetamide were in error unless one used charges appropriate for the two different conformations. These charges differed by as much as 0.24e, which illustrates the amount of charge that can shift around within the molecule through intramolecular inductive effects as conformation changes. The error incurred by neglecting the difference in charges between the two configurations was approximately 1 kcal/mol in water solution (the difference in energy between the configurations might be considerably more in the gas phase in the absence of dielectric screening, which weakens the effect of the Coulomb potential).

Reynolds, Essex and Richards [73] noted in free energy perturbation calculations of ethanol–propanol in water that the free energy of solvation difference between ethanol and propanol was in error if one did not take the difference in charges between conformations into account. They concluded that this conformational dependence of partial charges was more important than the question of method used for determining the charges. In another study, the same authors found that more accurate free energy differences were obtained if one determines the elec-

trostatic potential energy function of the molecule for several conformations from *ab initio* calculations, and then fits the charges to an average potential surface using Boltzmann weighting [74]. Their data shows that the dipole moment of a molecule varied too much from conformation to conformation when constant charges were used (the through-space inductive shifting of charge within a molecule would tend to minimize the dipole moment). Faerman and Price [75] and Price and Stone [35] calculated the electrostatic potential for different conformations of model peptides and concluded that even the more accurate distributed multipole analysis (DMA) was not invariant to changes in configuration. Similar conclusions were reached in a related study by some of the same researchers [76]. Another study noted similar conformational variation, but found that DMA charges were much more invariant than simple atomic point charges [77].

Urban and Famini [78] calculated partial atomic charges for dopamine by the electrostatic potential fitting method in six different conformations using *ab initio* calculations with two different basis sets. They found charges varied by as much as 0.4e, and that the variation was larger in the higher quality 6–31G* basis set. The differences between molecular mechanical steric energies using the six sets of charges was large, with a standard deviation of 3–5 kcal/mol. They also discouraged the Boltzmann weighting idea of Reynolds, Essex and Richards partly because the charge variation is so large as conformation changes that any single set of charges cannot adequately give the torsional energy profile properly. Urban and Famini concluded that this charge variation with conformation needs to be "adequately modeled in force field calculations." Stouch and Williams [79] similarly found great variations of atomic charge when fitting the electrostatic potential of eleven conformations of a model lipid. Dasgupta *et al.* encountered similar variations in dimethoxyethane [80]. Cornell *et al.* discuss conformational dependence of charges and its consequences [81]. Colonna and Evleth [82] also noted, although from a point of view of taking the polarizability into account, the conformational dependence of the electrostatic interaction.

One question that we have not yet addressed is the preferred method of determining partial charges. When one uses *ab initio* quantum chemical or semiempirical calculations, as many have done, there is a further ambiguity in that there are several ways to extract charges from wavefunctions. Some of the algorithms already available for obtaining charges are: Mulliken population analysis [64], natural orbital analysis [65], and fitting the charges to agree with the calculated electrostatic potential [66,69,80,81,83,84]. Other algorithms have been developed [21,85,86]. Some authors have compared the molecular electrostatic potential obtained from different levels of theory [87,88], basis set sensitivity [19,71,77,78,83,87] and the charges obtained using different fitting algorithms [84,89]. Nevertheless, a partial atomic charge is not a measurable quantity that can be found to be either right or wrong, because it is not possible to rigorously represent a continuous electron density distribution by a finite set of point charges. Thus, the question of method is really an empirical one to be answered by which method, when combined with the other

force field parameters with which they are optimized, give the best agreement with experiment and high quality *ab initio* calculations. Atomic point charges must be chosen both to optimally reproduce the forces that exist within a molecule between its atoms (intramolecular interactions) in order to determine conformational preference and also to reproduce forces between different molecules (intermolecular interactions) for condensed phase simulations. It is assumed (or at least hoped) that the same atomic partial charges can successfully model both intramolecular and intermolecular interactions [90], although this question has not been critically evaluated.

Stouch and Williams [91] determined from statistical analysis of fitting the molecular potential energy function for several conformations of a model lipid with atomic point charges that only the charges on the surface atoms are well determined by these fits. Charges on embedded atoms are not well determined in the fits, and most of the conformational variation of charges, as determined by fitting to the calculated electrostatic potential from the different conformations, comes from the fact that these interior charges are not well-determined. Investigators in Kollman's group have also noted this characteristic of charge fits and developed the RESP method to constrain certain charges [81,84]. Some might treat this fact as a reason to disregard the conformational variation of charge as due to statistical noise; however, we claim it as a warning that atomic charges must be treated carefully when considering intramolecular interactions. When atomic charges are fitted to the molecular electrostatic potential (recently, the most common method) in order to accurately model intermolecular forces, some of the charges which are important for intramolecular interactions are not necessarily well-determined. Thus, the relative conformational energies of the molecule (and thus the molecular recognition capability) which can be largely determined by the interaction of those charges is not well-determined.

The technique of fitting the charges to an electrostatic potential which is a Boltzmann weighted average of the electrostatic potential of several conformations has some advantage in the problem of ill-determined charges since different atoms may be near the surface in different conformations [84]. A disadvantage of Boltzmann averaging is that partial atomic charges determined this way are temperature dependent. In addition, we object to this procedure on physical grounds. Consider two conformations of 18-crown-6, one with an oxygen directed away from the ring, and another with the same oxygen atom pointed toward the cavity. The position of the oxygen atom in the first conformation may be occupied by a hydrogen atom in the second conformation. It is difficult to see what physical meaning the charge on the hydrogen atom has when it is adjusted to produce the field due partially to the presence of the oxygen atom at that position part of the time. A charge determined in this way is almost certainly inappropriate for intramolecular interactions. In addition, and especially for cyclic or floppy molecules, it may be difficult to identify the orientation of the molecule from one conformation to another in order

to unambiguously define which parts of the electrostatic potential surface should be averaged together.

One proposed method for determining partial atomic charges uses only the intramolecular electrostatic potential [86]. This method has not been thoroughly tested to see if the partial atomic charges are conformationally dependent, if so whether the variation is physically reasonable, or whether the intermolecular potential derived from intramolecularly determined charges is sufficiently accurate for molecular dynamics simulations.

There are at least three different physical effects that give rise to variations in partial atomic charges. The through-space inductive effect of electron repulsion (or attraction for electropositive regions) has been discussed. Williams [70] lists two (possibly) additional effects: hyperconjugation and polarization. When torsion angles adjacent to a double, triple or aromatic bond change, hyperconjugation describes the electron redistribution which may occur through interaction of the π orbitals. This charge redistribution needs to be accounted for in the partial atomic charges. Hyperconjugation will affect partial atomic charges, the torsional potential, as well as stretching and bending parameters. However, since 18-crown-6 is saturated, we judge the effect of hyperconjugation to be negligible in our calculations. Polarization describes the redistribution of charge that is not due to hyperconjugation. Both through-space and through-bond polarization can be identified. Through-space polarization could model part of the inductive effect previously described, although a complicated model of polarizability would be needed to describe transfer of charge across more than one bond as seen in Tables II–III. A simpler polarizability model would be that through which a dipole moment is induced on a single atom due to a nearby, charged, non-bonded atom. Several researchers have proposed methods to incorporate effective atomic polarizabilities to include such effects [27,55,82,92–96]. Atomic polarizability produces induced dipole moments, which have been shown to be important DMA analysis [35,75–77,83]; however, this effect would not change the partial atomic charges (monopoles) within the model.

It is sometimes argued [34,80,84] that one must insist on conformationally invariant charges for molecular dynamics simulations in order to minimize the complexity of these computationally demanding calculations. Almost all the recent research effort on the determination of partial charges has focused on duplicating intermolecular forces that are important in molecular dynamics simulations. The hope is that the intramolecular effects of charge variation with conformation can be built into the torsional part of the force field (of course, the transferability of such a torsional potential would be questionable) [81]. If the charges fitted to the molecular electrostatic potential do a good job of approximating the intermolecular interaction (as they should), then this philosophy is justified. Yet molecular recognition depends also on intramolecular interactions – specifically the relative energies of different conformations. If a fast, reliable method of computing partial atomic charges based on electronegativities, Coulomb integrals, and polarizabilities can be developed

from an empirical point of view, then the intramolecular problem would be much simplified. We feel that methods based on electronegativity, like the QEq algorithm, have potential usefulness since we found that relative conformational energies were particularly sensitive to the charge difference between oxygen and carbon for which electronegativity is a natural parameter. There is some indication that an electrostatic model that uses atomic dipoles (and possibly quadrupoles) might be advantageous [35,75–77,83]. If the hoped-for algorithm was sufficiently fast, it could be useful in molecular dynamics simulations as well.

Can conformationally dependent partial atomic charges be successfully incorporated into molecular mechanics force fields? For hydrocarbons, the electrostatic interaction is not dominant because H—C bonds are not strongly polar. Previous parameterizations for hydrocarbons have already demonstrated their utility and accuracy. For molecules with only one highly polar functional group, the same conclusions should be applicable. The importance of conformationally dependent charges should be high for molecules like polyethers, polyamines, and corresponding polymers. If suitable conformationally-dependent, partial atomic charges (or charges and dipoles, etc.) can be obtained from an empirical algorithm, then the other parameters in the force field will need to be refitted because a new set of electrostatic interactions requires that the other force field parameters be optimized specifically for those charges. This is especially true of van der Waals parameters and torsion potentials.

4. Conclusions

We have demonstrated that the electrostatic interactions as currently treated by molecular mechanics force fields are the most important determiner of the relative conformational energies of 18-crown-6. We have demonstrated that common, off-the-shelf molecular mechanics methods can give very different predictions for relative conformational energies depending on the choice of force field and choice of method for calculating the partial atomic charges. We demonstrated the extreme sensitivity of conformational energies for 18-crown-6 to small perturbations of the partial atomic charges.

The most important warning coming from this work is that one should not blindly couple an existing force field with a method for computing partial atomic charges for which the force field was not optimized. Figure 3 clearly shows that conformational ordering is extremely sensitive to small changes in the charges. Relative energy errors of several kcal/mol can be expected from charge determination errors of 0.05e. This sensitivity is enough to destroy confidence in the predictions of any force field that is not carefully optimized.

Examination of both semiempirical and *ab initio* quantum chemical wavefunctions suggests that partial atomic charges assigned to each atom can significantly change from conformation to conformation. These changes are of the same magnitude that we found changed the ordering of conformations in the perturbation

analysis. Thus, we would argue that dynamic charge assignment is essential to obtain meaningful results from molecular mechanics calculations of molecules with multiple polar groups. An empirical method like the charge equilibration algorithm [47] which includes electronegativity differences and Coulomb integrals, but generalized to include polarization effects, might be successful at this task and still be sufficiently fast to be usable in molecular mechanics of moderately large molecules. Researchers who are familiar with this problem should recognize the importance of this effect and seek to develop such a method.

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