

Calculation of the Free Energy of Solvation for Neutral Analogs of Amino Acid Side Chains

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Abstract: The ability of the GROMOS96 force field to reproduce partition constants between water and two less polar solvents (cyclohexane and chloroform) for analogs of 18 of the 20 naturally occurring amino acids has been investigated. The estimations of the solvation free energies in water, in cyclohexane solution, and chloroform solution are based on thermodynamic integration free energy calculations using molecular dynamics simulations. The calculations show that while the force field reproduces the experimental solvation free energies of nonpolar analogs with reasonable accuracy the solvation free energies of polar analogs in water are systematically overestimated (too positive). The dependence of the calculated free energies on the atomic partial charges was also studied.

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Introduction

Computer simulation is increasingly being used to understand and predict properties and processes within biomolecular systems at an atomic level. These include protein and peptide folding, the aggregation of lipids and surfactants, and the binding and recognition of ligands by their receptors, to name just a few.¹ A common feature of many of these biophysical processes is the partitioning of specific functional groups between different environments. For example, during folding, amino acids within a protein must partition between a highly polarizable aqueous environment and the predominantly hydrophobic interior of the protein. In the same manner, the binding of an inhibitor to a protein receptor depends on the free energy associated with the transfer of the ligand to be bound (and the amino acids lining the pocket within protein) from water to a less polar environment. A critical requirement of any molecular model or force field used for biomolecular simulations is, therefore, to correctly reproduce not only the conformational properties of the molecules involved but also the solvation and partitioning behavior of specific functional groups in, and between, different environments.^{2–4}

The most direct means to validate the partitioning properties of molecular force fields is to compare results from simulations with the available experimental data; in this case, by comparing partition constants (or distribution coefficients) for a series of solutes between water and a variety of less polar solvents. This is not possible for large biomolecular systems. It is possible for smaller

systems, such as analogs of amino acids for which experimental data is available in the literature.^{5,6}

In this article we use free energy calculations^{7,8} to investigate the ability of the GROMOS96 force field (43a2)^{9,10} to reproduce the solvation free energies in water, in cyclohexane solution, and in chloroform solution for the series of neutral amino acid analogs studied by Wolfenden and coworkers.^{5,6} The partition constants between water and cyclohexane as well as between water and chloroform are also evaluated.

Computational Techniques

The compounds investigated in this study were simple organic analogs of 18 of the 20 naturally occurring amino acids. Analogs of glycine and proline are not represented. Each analog was derived from the corresponding amino acid by truncating the side chain of the amino acid at the C_β and the replacement of the C_α by a hydrogen. The new terminal CH_n group was treated as a united atom in line with the rest of the force field. The names of the compounds investigated together with the corresponding amino acid residues are listed in Tables 1 and 2. All the compounds were investigated in their neutral form. This allowed a direct comparison with the experimental data of Radzicka et al.⁵ Simulations

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Table 1. Experimental⁵ and Calculated Solvation Free Energies (kJ mol⁻¹) at $T = 293$ K for Neutral Analogs of 18 of the 20 Naturally Occurring Amino Acids in Cyclohexane and Chloroform.

Compound	Cyclohexane		Chloroform	
	Exp	Calc	Exp	Calc
Methane (Ala)	0.6	0.8 ± 0.6		
Propane (Val)	-8.5	-6.7 ± 0.9		
Butane (Ile)	-11.4	-13.0 ± 1.5		
Isobutane (Leu)	-10.9	-9.8 ± 1.5		
Methylimidazole (His)	-23.4	-21.1 ± 1.0		
Methylindole (Trp)	-33.8	-35.9 ± 2.8	-36.8	-39.6 ± 1.4
p-Cresol (Tyr)	-24.6	-28.3 ± 1.2		
Toluene (Phe)	-15.4, -19.6	-25.2 ± 1.0	-15.9	-25.1 ± 1.3
Methanol (Ser)	-6.9	-3.5 ± 0.9	-13.8, -13.2	-6.1 ± 0.7
Ethanol (Thr)	-9.5	-7.8 ± 0.8		
Acetamide (Asn)	-12.6	-14.3 ± 1.0	-28.7	-22.2 ± 1.0
Propionamide (Gln)	-15.8	-19.3 ± 0.9	-30.8	-25.5 ± 0.7
n-Butylamine (Lys)	-16.4	-16.8 ± 1.8	-23.5	-19.3 ± 1.5
n-Propylguanidine (Arg)	-20.6	-24.0 ± 1.8		
Acetic acid (Asp)	-9.2	-15.6 ± 1.1	-17.5	-17.4 ± 1.4
Propionic acid (Glu)	-13.9	-18.6 ± 1.1	-21.9	-20.5 ± 1.2
Methanethiol (Cys)	-10.3	-7.9 ± 0.8		
Methyl-ethylsulfide (Met)	-15.8	-14.4 ± 1.3		

The calculated values are reported together with the associated error.

were performed in four different environments: in vacuum, in water solution, in cyclohexane solution, and in chloroform solution. The Simple Point Charge (SPC) model¹¹ was used to describe the water. Chloroform was described by the standard rigid model

implemented in the GROMOS96 (43a2) force field.¹² Cyclohexane was also described using standard parameters within the 43a2 force field. The amino acid analogs were described using the force field parameters of the corresponding amino acids. The only allowance

Table 2. Experimental and Calculated Hydration Free Energies (kJ mol⁻¹) at $T = 293$ K for Neutral Analogs of 18 of the 20 Naturally Occurring Amino Acids.

Compound	Exp		Calc		
	Reference 6	Reference 23	GROMOS96	Reference 24	Q1.1
Methane (Ala)	8.1	8.4	9.2 ± 0.6	8.0 ± 1.3	
Propane (Val)	8.2	8.2	10.7 ± 1.2	9.0 ± 2.2	
Butane (Ile)	8.8	8.7	10.3 ± 1.4	7.7 ± 2.2	
Isobutane (Leu)	9.4	9.7	10.4 ± 1.1	10.4 ± 2.2	
Methylimidazole (His)	-42.1		-27.4 ± 1.2		-36.0
Methylindole (Trp)	-24.3		-12.3 ± 1.9		-15.8
p-Cresol (Tyr)	-25.2	-25.7	-22.4 ± 2.2		-30.2
Toluene (Phe)	-3.1	-3.7	3.4 ± 1.3		2.0
Methanol (Ser)	-20.8	-21.4	-14.1 ± 0.9		-20.6
Ethanol (Thr)	-20.1	-21.0	-13.6 ± 1.1		-20.5
Acetamide (Asn)	-39.9	-40.6	-18.8 ± 1.7		-26.5
Propionamide (Gln)	-38.7		-18.7 ± 2.0		-26.7
n-Butylamine (Lys)	-18.0		-15.5 ± 2.2		-22.2
n-Propylguanidine (Arg)	-44.8		-30.1 ± 2.4		-40.1
Acetic acid (Asp)	-27.5	-28.1	-18.2 ± 1.1		-24.0
Propionic acid (Glu)	-26.6	-27.1	-16.2 ± 1.1		-21.8
Methanethiol (Cys)	-5.1	-5.2	5.5 ± 1.0		5.3
Methyl-ethylsulfide (Met)	-6.1		9.0 ± 1.9		

The calculated values are reported together with the associated error. GROMOS96 refers to values calculated using the GROMOS96 (43a2) force field. Q1.1 refers to values calculated after all charges were increased by 10%.

made for the effect of the substitution of the C_α by a hydrogen was the change in the van der Waals parameters of the C_β .

The simulations were performed using the GROMACS (GROningen Machine for Chemical Simulation) package^{13–15} (version 3.0) at constant temperature and pressure in a periodic cubic box with an edge length of approximately 4.5 nm. This corresponded to approximately 3000 water molecules or alternatively 500–600 cyclohexane or chloroform molecules at the experimental density. The nonbonded interactions were evaluated using a twin range cutoff of 0.8 and 1.4 nm. Interactions within the shorter range cutoff were evaluated every step, whereas interactions within the longer cutoff were updated only every five steps, together with the pair list. To obtain an isothermal–isobaric ensemble at 293 K, a leap-frog stochastic dynamics integrator¹⁶ was used to integrate the equations of motion. The inverse friction constant was set to 0.2 ps. The pressure was maintained constant by coupling to a reference pressure of 1 bar using the Berendsen barostat.¹⁷ For the simulations in water the coupling time was set to 1.0 ps, and the isothermal compressibility was set to $4.6 \cdot 10^{-5} \text{ bar}^{-1}$. For cyclohexane and chloroform the coupling times and isothermal compressibilities were 2.0 and 5.0 ps, and $11.2 \cdot 10^{-5} \text{ bar}^{-1}$ and $9.98 \cdot 10^{-5} \text{ bar}^{-1}$, respectively. The bond distances and bond angles of water were constrained using the SETTLE algorithm.¹⁸ All other bond distances and the bond angles in chloroform were constrained using the LINCS algorithm.¹⁹ The time step used in the simulations was 0.002 ps. A 200-ps simulation was performed to equilibrate each of the systems before the start of the free energy calculations.

Free-Energy Calculations

The difference in free energy between two states of a system can be determined using the coupling parameter approach in conjunction with the thermodynamic integration (TI) formula⁸:

$$\Delta F_{AB} = \int_{\lambda_A}^{\lambda_B} \left\langle \frac{\partial H(\lambda)}{\partial \lambda} \right\rangle_{\lambda'} d\lambda' \quad (1)$$

In this approach the Hamiltonian H is made a function of a coupling parameter, λ . The λ -dependence of the Hamiltonian defines a pathway that connects two states of the system denoted by A and B. One approach to solve eq. (1) is to evaluate the ensemble average at a number of discrete λ -points by performing separate simulations for each chosen λ -point. The integral can then be determined numerically.

If the difference between two states of the systems is small, an alternative to the TI formula or other multistep approaches is to compute the difference in free energy between two states directly using the free-energy perturbation formula⁸:

$$\Delta F_{AB} = -k_B T \ln \langle e^{-[H(\lambda_B) - H(\lambda_A)]/k_B T} \rangle_{\lambda_A} \quad (2)$$

where k_B is the Boltzmann constant, and T is the temperature. Using eq. (2), the free energy is calculated from the relative probability a given configuration sampled in an ensemble at λ_A

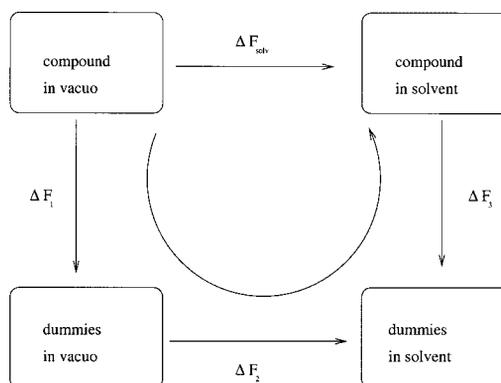


Figure 1. Thermodynamic cycle for the determination of solvation free energies.

being sampled in the ensemble at λ_B . To obtain convergence, significant overlap of the low energy regions of the two ensembles is required.

Solvation Free-Energy Calculations

The solvation free energy, ΔF_{solv} , is the work required to transfer a molecule from the gas phase into solution and was calculated according to the thermodynamic cycle shown in Figure 1. In Figure 1, ΔF_1 is the work required to remove all the internal nonbonded interactions in the compound *in vacuo*. This was achieved by gradually mutating all atoms in a given compound (state A) into “dummy” atoms (state B). In this context a “dummy” atom is an atom for which the nonbonded interaction, namely the Lennard–Jones and electrostatic interaction parameters with all other atoms have been set to zero. The bonded interactions within the molecule and the masses of the individual atoms were kept unchanged. ΔF_2 is the work required to transfer the dummy solute from vacuum to the solvated phase. As the molecule does not interact with the rest of the system, and the available volume is the same, this term is effectively zero. ΔF_3 is the work required to remove the solute–solvent and the solute–intramolecular interactions. This was again achieved by gradually mutating all atoms in a given compound (state A) into “dummy” atoms (state B).

The nonbonded interactions between the initial state (A, the interacting solute) and the final state (B, the dummy solute) were interpolated using a soft-core potential $V^{\text{nb}}(r)$.²⁰

The specific nonbonded interaction function between two atoms, i and j , implemented in the GROMACS simulation package is:

$$V_{ij}^{\text{nb}}(r) = (1 - \lambda)V_{ij}^{\text{A}}(r) + \lambda V_{ij}^{\text{B}}(r) \quad (3)$$

where:

$$V_{ij}^{\text{A}}(r) = \left(\frac{C_{ij}^{(12)}}{(r_{ij}^{\text{A}})^{12}} - \frac{C_{ij}^{(6)}}{(r_{ij}^{\text{A}})^6} \right) + f \frac{q_i q_j}{r_{ij}^{\text{A}}} \quad (4)$$

$$V_{ij}^B(r_{ij}^B) = \left(\frac{C_{ij}^{(12)}}{(r_{ij}^B)^{12}} - \frac{C_{ij}^{(6)}}{(r_{ij}^B)^6} \right) + f \frac{q_i q_j}{r_{ij}^B} \quad (5)$$

$$r_{ij}^A = (\alpha(\sigma_{ij}^A)^6 \lambda^2 + r_{ij}^6)^{1/6} \quad (6)$$

$$r_{ij}^B = (\alpha(\sigma_{ij}^B)^6 (1 - \lambda)^2 + r_{ij}^6)^{1/6} \quad (7)$$

V_{ij}^A and V_{ij}^B are the potentials describing the nonbonded interactions between two atoms at a distance of r_{ij} in the state A ($\lambda = 0$) and in the state B ($\lambda = 1$), respectively. $C_{ij}^{(12)}$ and $C_{ij}^{(6)}$ are the van der Waals parameters for the repulsion and the dispersion energy terms for the interaction, respectively, f is equal to $1/(4\pi\epsilon_0)$, and q_i and q_j are the atomic charges. α is the soft-core parameter, which essentially controls the height of the potential around $r_{ij} = 0$. A value of 1.51 was used. The σ_{ij}^A , σ_{ij}^B parameters correspond to $(C_{ij}^{(12)}/C_{ij}^{(6)})^{1/6}$ or were assigned a predefined value of 0.3 nm in cases where $C_{ij}^{(6)}$ or $C_{ij}^{(12)}$ was zero.

For each system the free energy associated with the removal of the nonbonded interactions was estimated using the TI formula [eq. (1)]. Separate simulations were performed at a number of λ -points (around 16) from $\lambda = 0$ (state A—solute) to $\lambda = 1$ (state B—dummy atoms). At each λ -point the system was first equilibrated for 50 ps and then data was collected for a further 150 ps. The averages of the derivatives, $\partial H(\lambda)/\partial \lambda$, at each of the λ -points [eq. (1)] were then integrated using the trapezoidal method to obtain ΔF_{AB} . The error in $\langle \partial H(\lambda)/\partial \lambda \rangle_\lambda$ was estimated using a block averaging procedure.^{21,22} The individual errors were integrated from $\lambda = 0$ to $\lambda = 1$ to yield the estimate of the total error in ΔF_{AB} given.

Results and Discussion

Solvation Free Energies and Partition Properties

In validation studies such as this, the choice of reference compounds and solvents is very important. Cyclohexane was chosen as the hydrophobic reference state not only because of its low dielectric constant but also because it contains very little water at saturation. Moreover, by comparing the free energies for the transfer of the chosen amino acid analogs from water to cyclohexane⁵ with the corresponding free energies for the transfer of the same analogs from the vapor phase to water,⁶ it is possible to infer equilibrium constants for the transfer of the analogs from the vapor phase directly to cyclohexane. Only neutral analogs were considered to be able to make a direct comparison with the experiment.

The partition constants between water and cyclohexane were evaluated for all the systems investigated. The partition constants between water and chloroform were calculated for those systems where the corresponding experimental data is available. Note that in both these cases it is implicitly assumed in the calculations that no water molecules partition into the more hydrophobic phase together with the amino acid analog. For chloroform this is likely to be a poor assumption in the case of some polar compounds.

The transformations during the free energy calculations from an interacting to a noninteracting ghost or dummy molecule were performed using a minimum of 16 λ points, which in most cases

was sufficient to ensure a smooth integrand. In cases where the curve to be integrated corresponding to eq. (1) showed an apparent discontinuity, the number of λ -points was increased as appropriate. To check that the value of the derivative at specific values of λ had converged, the sampling time at a number of chosen λ -points was extended to 300 ps. In no case was a significant difference in the final free energy value observed on extending the calculations.

A stochastic integrator was used to integrate the equations of motion in this study. This integrator was used to prevent an accumulation of translational and rotational kinetic energy in the compound being mutated as it became uncoupled from the rest of the system, a known artifact of the Berendsen thermostat. During the transformation from $\lambda = 0$ to $\lambda = 1$ using the stochastic integrator the rotational kinetic energy of the compounds studied in solution and *in vacuo* was around 3.2–4.0 kJ mol⁻¹. These values are consistent with the equipartition theorem, namely that the rotational kinetic energy of a molecule is equal to $\frac{3}{2} k_B T$. Using a classical molecular dynamics integration scheme together with a Berendsen thermostat under the same conditions resulted in a much higher value of rotational kinetic energy (around 30 kJ mol⁻¹) as λ approached 1 and the rotational and translational degrees of freedom of the solute became uncoupled from the rest of the system.

The experimental⁵ and the calculated solvation free energies of the compounds in cyclohexane solution and in chloroform solution are reported in Table 1. The experimental^{6,23} and the calculated hydration free energies are reported in Table 2. The estimated statistical error in the free energies (Tables 1 and 2) is in the range of 0.6 to 2.8 kJ mol⁻¹.

Table 1 shows that in general, the calculated solvation free energies in cyclohexane and chloroform solution are in good agreement with the experiment. Compounds containing sp² carbons have, however, slightly lower than expected values. Among these the values for toluene show the largest discrepancy with experiment.

The hydration free energies are reported in Table 2. The calculated values for all the hydration free energies of the aliphatic compounds and for cresol are close to the experimental data. The values for the aliphatic compounds are also in agreement with those obtained previously by Daura et al.²⁴ (Table 2).

For the other amino acid analogs the calculated values for the hydration free energy are systematically higher than the experimental values. The hydration of these compounds as described by the molecular model is less favorable than that is observed experimentally. The qualitative largest difference between the calculations and experiment is observed for the analogs of the amino acids cysteine, methionine and phenylalanine. Experimentally, these compounds have a negative hydration free energy, whereas the simulations predict these molecules prefer to be in the gas phase as opposed to in water.

From the calculated solvation free energy, the distribution coefficient $\log K$ was evaluated according to the following relation:

$$\Delta F_{\text{solv} \rightarrow \text{wat}} = -2.303RT \log K \quad (8)$$

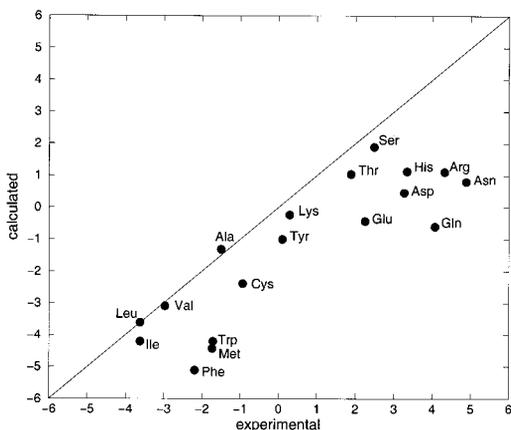


Figure 2. Water/cyclohexane distribution coefficients, $\log K$ at $T = 293$ K.

where $\Delta F_{\text{solv} \rightarrow \text{wat}}$ is the change in free energy passing from a low dielectric solvent (cyclohexane or chloroform) to water, R the gas constant, and T the absolute temperature.

The calculated partition constants $\log K$ are plotted against the available experimental data⁵ in Figures 2 and 3. The results for the water/cyclohexane partition constants are plotted in Figure 2, and for the water/chloroform partition constants the results are plotted in Figure 3. The points in both figures are labeled with the name of the corresponding amino acid.

Figures 2 and 3 show that for the majority of the analogs investigated the force field is able to reproduce the correct trend between water and the less polar solvents. Moreover, for the analogs of alanine, leucine, valine, isoleucine, threonine, and serine the calculated partition constants are very close to the experimental values (Fig. 2). This is also observed for the analog of serine in the mixture water/chloroform (Fig. 3). However, the analogs of glutamate, glutamine, tyrosine, and lysine, prefer cyclohexane and chloroform (Figs. 2 and 3) over water, which is in contradiction to their experimental observed chemical-physical behavior. The analog of asparagine also prefers chloroform over water, which is opposite to that experimentally observed.

Dependence of the Calculated Hydration Free Energy on the Atomic Charges

Clearly, the results indicate that the partial atomic charges used to model the electrostatic interactions between water and the polar analogs are systematically too low in the GROMOS96 force field. Therefore, the effect of scaling the atomic charges on the calculated free energy values was investigated. This was performed to have an indication of the dependence of the hydration free energies on the atomic charges and not to propose a refined force field.

The GROMOS96 atomic charges for all the atom types were increased by 10%. The new set of parameters will be referred to as Q1.1. New estimates for hydration free energies $\Delta F_{\text{wat}}^{\text{new}}$ were calculated from the following relation:

$$\Delta F_{\text{wat}}^{\text{new}} = \Delta F_{\text{wat}} + \Delta F^{\text{Q1.1}} \quad (9)$$

where ΔF_{wat} is the hydration free energy calculated using the standards GROMOS96 force field as described earlier (Table 2). $\Delta F^{\text{Q1.1}}$ is the sum of the differences in ΔF_1 and ΔF_3 due to the increase in the atomic charges from the standard GROMOS96 values (state A) to the scaled charges (state Anew) for each of the compounds investigated. Note that no mutation was performed for the cases where the partial atomic charges were zero (i.e., the analog of methionine).

The difference in free energy between state A and state Anew was calculated using the perturbation formula, assuming that the difference between A and Anew was small. Equation (2) was applied using the 150 ps simulations performed in the state A for data collection.

The value of $\Delta F_{\text{wat}}^{\text{new}}$ was estimated according to relation (9) for all the analogs that contained atom types with net atomic charges. The values of $\Delta F_{\text{wat}}^{\text{new}}$, labeled Q1.1 in Table 2, show that the hydration free energy of a compound using the set Q1.1 is more favorable than when using the standards GROMOS96 force field, as expected. For many of the compounds investigated increasing the charge by the 10% is sufficient to shift the hydration free energy close to the experimental values, as shown in Figure 4. For the analogs of tyrosine and lysine the 10% change resulted in an overestimation of the hydration free energies. For cysteine scaling the charge had little to no effect. This is to be expected as the partial atomic charge in the GROMOS96 force field on the hydrogen and the sulfur are only $+0.064e$ and $-0.064e$, respectively.

Conclusions

In general, the GROMOS96 (43a2) force field is able to reproduce, with reasonable accuracy, the solvation free energies of the compounds studied in a hydrophobic environment (cyclohexane), and in a low polar solvent (chloroform). In addition, the calculated free energies of hydration of the analogs of the hydrophobic amino acids alanine, valine, isoleucine, and leucine are close to the available experimental values. These values are primarily determined by the Lennard-Jones parameters of the force field, which

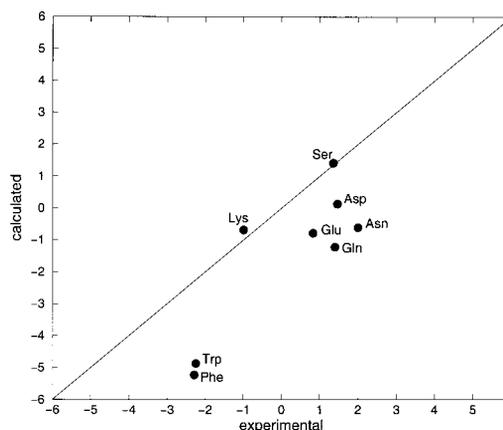


Figure 3. Water/chloroform distribution coefficients, $\log K$ at $T = 293$ K.

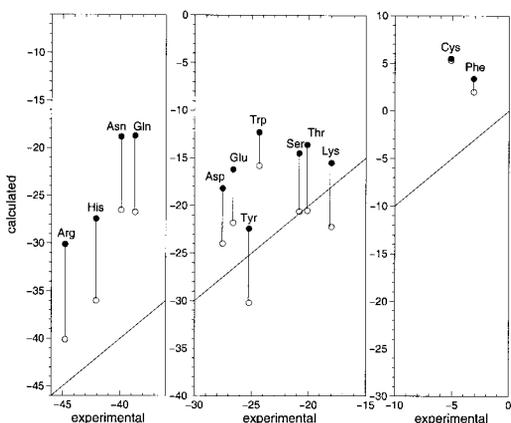


Figure 4. Hydration free energies (kJ mol^{-1}) at $T = 293 \text{ K}$: experimental vs. calculated using the GROMOS96 force field (43a2) (●) and the Q1.1 force field (○).

mimic the van der Waals interactions between the solute and solvent.

The calculated values for the hydration free energies of analogs of the polar amino acids (His, Ser, Thr, Asn, Gln, Lys, Arg, Asp, Gln) do not agree as well with the experiment. The force field systematically overestimates the free energy of hydration. The values are too positive, indicating that the analogs show less affinity to water than expected by on average 11.7 kJ mol^{-1} . This is a consequence of the partial charges, and hence, the electrostatic interactions in the GROMOS force field being kept as low as possible. The average discrepancy between calculated and experimental hydration free energies decreases from 11.7 kJ mol^{-1} to 4.5 kJ mol^{-1} if the atomic charges are increased by 10%.

In terms of the overall properties of the force field it should be stressed that the analogs of arginine, lysine, aspartate, and glutamate were neutral. This allowed a direct comparison to the available experimental data. At physiological pH, however, these side chains almost always carry a net charge, which will dominate the interactions.

In contrast to the polar aliphatic side-chain analogs, the hydration free energies of the analogs of the aromatic amino acids (Phe, Trp, and Tyr) show reasonable agreement with experiment especially for the analogs of phenylalanine and tyrosine. The analogs of the sulphur amino acids, cysteine, and methionine, clearly show anomalous behavior in water yielding positive hydration free energies.

There is some compensation of errors within the force field in the calculation of partition behavior between water and less polar environments. The force field reproduces the correct trend for all the analogs except for analogs of phenylalanine, lysine, glutamine, and glutamate.

The fact that the results in chloroform are closer to the experimental data than those in water indicates that one set of charges may not be appropriate for all environments, and that the inclusion of a polarisation term in the force field may be required.

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