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Use of a QM/MM-Based FEP Method to Evaluate the Anomalous Hydration Behavior of Simple Alkyl Amines and Amides: Application to the Design of FBPase Inhibitors for the Treatment of Type-2 Diabetes

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Supporting Information

ABSTRACT: Standard molecular mechanics (MM) force fields predict a nearly linear decrease in hydration free energy with each successive addition of a methyl group to ammonia or acetamide, whereas a nonadditive relationship is observed experimentally. In contrast, the non-additive hydration behavior is reproduced directly using a quantum mechanics (QM)/MM-based free-energy perturbation (FEP) method wherein the solute partial atomic charges are updated at every window. Decomposing the free energies into electrostatic and van der Waals contributions and comparing the results with the corresponding free energies obtained using a conventional FEP method and a QM/MM method wherein the charges are not updated suggests that inaccuracies in the electrostatic free energies are the primary reason for the inability of the conventional FEP method to predict the experimental findings. The QM/MM-based FEP method was subsequently used to evaluate inhibitors of the diabetes drug target fructose-1,6-bisphosphatase adenosine 5′-monophosphate and 6-methylamino purine riboside 5′-monophosphate. The predicted relative binding free energy was consistent with the experimental findings, whereas the relative binding free energy predicted using the conventional FEP method differed from the experimental finding by an amount consistent with the overestimated relative solvation free energies calculated for alkylamines. Accordingly, the QM/MM-based FEP method offers potential advantages over conventional FEP methods, including greater accuracy and reduced user input. Moreover, since drug candidates often contain either functionality that is inadequately treated by MM (e.g., simple alkylamines and alkylamides) or new molecular scaffolds that require time-consuming development of MM parameters, these advantages could enable future automation of FEP calculations as well as greatly increase the use and impact of FEP calculations in drug discovery.

Free-energy perturbation (FEP) is widely recognized as the most accurate computational method[1−3] for calculating relative solvation[4] and binding[5,6] free-energy differences. The accuracy of conventional FEP calculations, while superior to other less time-consuming methods,[7] still relies on a molecular mechanics (MM) force field and the accuracy of the equations and parameters that constitute the force field. While force field parameters are available for numerous atom types and molecular structures, drug candidates often contain new scaffolds that are inadequately represented by the default set of generalized force field parameters or parameters automatically derived from an extrapolation algorithm. Consequently the user must develop and input the corresponding MM parameters, and this process is not only time-consuming but often limited by the absence of relevant experimental data.[8]

To improve the accuracy of the FEP method and provide a means of semiautomating FEP calculations, we developed a quantum mechanics (QM)-based FEP method several years ago.[9] Prior work by Warshel and co-workers[10] had successfully used the coupling of QM and MM methods to calculate free-energy profiles of enzymatic reactions, whereas our efforts were focused on a method for calculating relative solvation[9] and binding free energy differences[11] between two solutes/ligands. Calculated results obtained using QM to treat the ligand and MM to treat the surrounding solvent and protein as well as the interactions between them were consistent with experimental data.

In this work, we used the QM/MM-based FEP method to calculate the absolute and relative differences in hydration free energy for simple alkylamines and alkylamides. Experimentally these molecules exhibit a rank order [NH_2CH_3 < NH_3 ≈ $NH(CH_3)_2$ < $N(CH_3)_3$] that differs from the upward trend in hydrophobicity expected for the successive addition of methyl groups.[12−14] This anomalous behavior has proven difficult to reproduce computationally using continuum and quantum-continuum methods[15] and standard MM force fields.[16] More recently, the relative hydration free-energy differences were reproduced using an all-atom[17] or united-atom force field,[18] but only after adjustments in the force field parameters (partial changes and Lennard-Jones parameters) were made. Calculations using conventional and QM/MM-based FEP methods were performed using procedures previously described.[6,9,11] In both cases, the λ-coupling

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Table 1. Relative Solvation Free Energies (in kcal/mol) of Simple Amines

transformation	$\Delta\Delta G$(QM)[a]	$\Delta\Delta G$(MM)[b]	$\Delta\Delta G$(E)[c]
$NH_3 \rightarrow NH_2Me$	-0.20 ± 0.4	0.50 ± 0.4	-0.26
$NH_2Me \rightarrow NHMe_2$	0.60 ± 0.4	1.50 ± 0.5	0.27
$NHMe_2 \rightarrow NMe_3$	1.40 ± 0.5	2.10 ± 0.6	1.06
$NH_3 \rightarrow NHMe_2$	0.35 ± 0.6	1.90 ± 0.6	0.01
$NH_3 \rightarrow NMe_3$	1.60 ± 0.7	3.60 ± 0.8	1.07

[a] Calculated using the ab initio QM/MM-based FEP method with HF/6-31G**/ESP-derived partial atomic charges updated at every window. [b] Calculated using a conventional FEP method with HF/6-31G**/ESP-derived partial atomic charges. [c] Values obtained from experimental data.[12−14]

method was used to transform solute A into solute B and the thread technique[6,11] was used to map structurally dissimilar molecules. In the conventional method, MM parameters were used to calculate MM energies and forces. In the QM/MM-based FEP method, ab initio QM [Hartree−Fock (HF)/6-31G*] was used to calculate the energies and forces for the ligand while MM was used to calculate the energies and forces for the solvent and protein as well as the interactions between them [see the Supporting Information (SI) for details].

We tested the ability of the ab initio QM/MM-based FEP method for several simple alkyl amine analogues by calculating the relative hydration free energies for the transformations $NH_3 \rightarrow NH_2Me$, $NH_2Me \rightarrow NHMe_2$, $NHMe_2 \rightarrow NMe_3$, $NH_3 \rightarrow NHMe_2$, and $NH_3 \rightarrow NMe_3$ using eq S14 and Figure S1 (see the SI).

As shown in Table 1, the relative hydration free energies calculated using the ab initio QM/MM-based FEP method [$\Delta\Delta G$(QM)] reproduced the anomalous behavior observed experimentally [$\Delta\Delta G$(E)].[12−14] In contrast but consistent with earlier reports,[15,16] the conventional FEP approach [$\Delta\Delta G$(MM)] predicted a progressive decrease in hydration free energy with each addition of a methyl substituent.

Like alkylamines, simple alkylamides exhibit similar anomalous behavior.[12,13] As shown in Table 2 and Figure 1, the QM/MM-based FEP method reproduced the experimental results whereas conventional FEP failed, just as previously reported by others.[16]

Closer agreement with experimental findings was also obtained (Table 2) with this method for two isomers, *trans*- and *cis*-*N*-methylacetamide, that surprisingly exhibit similar hydration free energies despite large differences in their permanent dipole moments.[12,13] Additional evidence for the robustness of this method was derived from the good agreement between the calculated and experimental absolute solvation free energies of NH_3 and NH_2Me (Table 3).

The ability of the QM/MM-based FEP method to reproduce the anomalous hydration behavior led to additional calculations comparing the conventional FEP method [$\Delta\Delta G$(MM)] with two variants of the QM/MM-based FEP method, one [$\Delta\Delta G$(QM′)] wherein the partial atomic charges were kept the same (consistent with the reported conventional FEP calculations[6,16]) and the other [$\Delta\Delta G$(QM)] wherein the charges were updated after every window. Separation of the electrostatic (ele) and van der Waals (vdw) contributions provided additional insight. The following results were obtained: for the $NH_3 \rightarrow NH_2CH_3$ transformation, $\Delta\Delta G$(MM) = 0.5 kcal/mol [0.3 (ele) + 0.2 (vdw)], $\Delta\Delta G$(QM′) = 0.35 kcal/mol [0.20 (ele) + 0.15 (vdw)], and $\Delta\Delta G$(QM) = −0.2 kcal/mol [−0.3 (ele) + 0.1 (vdw)]; for the $NH_3 \rightarrow N(CH_3)_3$ transformation, $\Delta\Delta G$(MM) = 3.6 kcal/mol [1.0 (ele) + 2.6 (vdw)], $\Delta\Delta G$(QM′) = 2.65 kcal/mol [0.85 (ele) + 1.80 (vdw)], and $\Delta\Delta G$(QM) = 1.60 kcal/mol [0.1 (ele) + 1.50 (vdw)]. In both cases, the results calculated using $\Delta\Delta G$(QM) gave excellent agreement with the experimental results (−0.2 vs −0.26 kcal/mol and 1.6 vs 1.07 kcal/mol), which suggests that the greater accuracy realized by $\Delta\Delta G$(QM) (Table S1) can largely be attributed to differences in the handling of the partial atomic charges and the inaccuracies introduced primarily into the electrostatic contribution to the free energy when the partial atomic charges of the solute are not updated throughout the transformation. Similar trends were observed in calculations involving transformations of simple amides (Table S1).

We next evaluated the potential importance of these findings in drug design by calculating the relative solvation and binding free energies of adenosine 5′-monophosphate (**AMP**) and 6-methylamino purine riboside 5′-monophosphate (**I**) (structures shown

Table 2. Relative Solvation Free Energies (in kcal/mol) of Simple Amides

transformation[a]	$\Delta\Delta G$(QM)[b]	$\Delta\Delta G$(MM)[c]	$\Delta\Delta G$(E)[d]
AC → MAC	-0.25 ± 0.4	1.70 ± 0.4	-0.40
MAC → DMAC	1.35 ± 0.4	1.10 ± 0.5	1.53
AC → DMAC	1.25 ± 0.6	2.60 ± 0.7	1.13
trans-MAC → *cis*-MAC	-0.30 ± 0.3	-0.60 ± 0.3	∼0.0

[a] Abbreviations: AC, acetamide; MAC, *N*-methylacetamide; DMAC, *N*,*N*-dimethylacetamide. [b] Calculated using the ab initio QM/MM-based FEP method and HF/6-31G**/ESP-derived partial atomic charges updated at every window. [c] Calculated using a conventional FEP method and HF/6-31G**/ESP-derived partial atomic charges. [d] Values obtained from experimental data.[12−14]

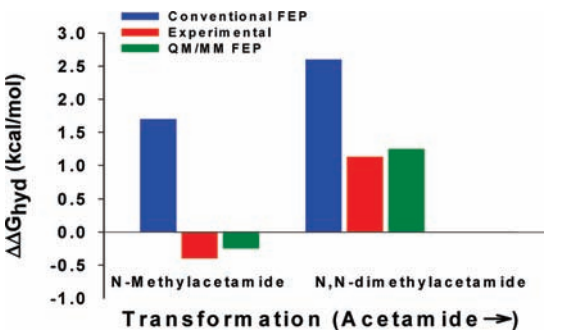

Figure 1. Relative solvation free energies of simple alkyl amide analogues.

Table 3. Solvation Free Energies (in kcal/mol) of Ammonia, Methylamine, and Water

molecule	$\Delta\Delta G$(QM)[a]	$\Delta\Delta G$(MM)[b]	$\Delta\Delta G$(E)[c]
NH_3	-4.10 ± 0.6	-4.05 ± 0.6	-4.30
NH_2Me	-4.35 ± 0.7	-3.65 ± 0.7	-4.60
H_2O	-6.40 ± 0.6	-6.15 ± 0.6	-6.30

[a] Calculated using the ab initio QM/MM-based FEP method and HF/6-31G**/ESP-derived partial atomic charges updated at every window. [b] Calculated using a conventional FEP method and HF/6-31G**/ESP-derived partial atomic charges. [c] Values obtained from experimental data.[12−14]

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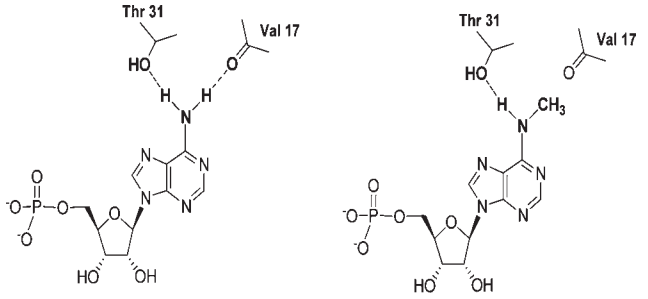

Figure 2. FBPase inhibitor structures and 6-amino hydrogen-bonding interactions with FBPase: (left) **AMP** and (right) 6-methylamino-substituted purine riboside monophosphate (**I**).

in Figure 2) complexed with fructose-1,6-bisphosphatase (FBPase) using both the conventional[6] and QM/MM-based FEP methods.[11] In the QM/MM-based FEP method, inhibitor gradients and energies were calculated at every MD step using AM1. Partial atomic charges were calculated[11] using the HF/6-31G**/electrostatic potential (ESP) method and updated at every window, while the rest of system (solvent + protein) was treated using the MM force field.[19] In the conventional FEP method, partial atomic charges were calculated using the HF/6-31G**/ESP method[11] only at the beginning of the MD simulations, and the system (solvent + inhibitor + protein) was treated using the MM force field.[19] The calculated relative solvation and binding free energies between **AMP** and **I** using the conventional and QM/MM-based FEP methods[6,11] were 2.0 ± 0.5 and 0.6 ± 0.5 kcal/mol, respectively, and 0.7 ± 0.6 and 1.7 ± 0.6 kcal/mol, respectively, whereas the experimental value is 1.5 kcal/mol. The relative binding affinity predicted using conventional FEP method[6] differed from the experimental finding by an amount consistent with the overestimated relative solvation free energies calculated for alkylamines. In contrast, the QM/MM-based FEP method accurately predicted the relative binding affinity, which supports the use of this method in drug design.

In summary, amines and amides represent functionality commonly found in drug candidates. Accordingly, the failure of earlier efforts to accurately calculate absolute and relative hydration free energies of simple alkylamines and alkylamides[16] raises concerns regarding possible inherent inaccuracies associated with these methods that could limit their ability to computationally discriminate between drug candidates. Inaccuracies can be rectified in some cases by extensive reparametrization of the force field,[17,18] but this requires time-consuming input by the user, who often either lacks the expertise or is limited by the absence of relevant experimental data. Moreover, the dependence on the user for ligand-specific input prevents automation of FEP-based calculations and therefore calculation throughput. The QM/MM-based FEP method offers a potential strategy for obtaining accurate free energies without these limitations and therefore may ultimately prove to be useful for integrating FEP calculations into drug discovery efforts focused on the identification and optimization of lead compounds.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Theoretical background, methodology, thermodynamic cycle−perturbation approach (Figure S1), and computational details related to the calculation of relative solvation free energies of alkylamine and alkylamide analogues and binding free energies of FBPase inhibitors (AMP analogues). This material is available free of charge via the Internet at http://pubs.acs.org.

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