Communication

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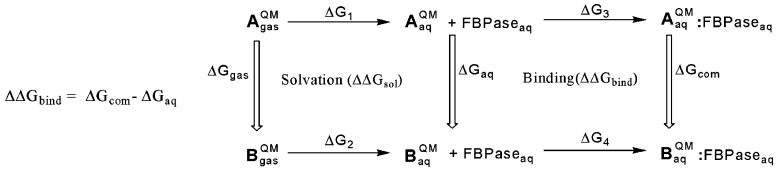

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Relative Binding Affinities of Fructose-1,6-Bisphosphatase Inhibitors Calculated Using a Quantum Mechanics-Based Free Energy Perturbation Method

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Advances in X-ray crystallography over the past two decades have produced an unprecedented number of high-resolution protein structures. Efforts to use structural information to shorten the drug discovery timeline have led to the development of various computational methods for calculating relative binding affinities.[1] Most of these methods use algorithms that rely on a multitude of approximations designed to increase computational speed and compound throughput through simplified treatment of entropy, solvent interactions, and/or molecular mechanics force fields describing binding energies. Virtual screening of large, computationally generated compound libraries using highly automated procedures has produced computational hits that proved to be valuable starting points for subsequent synthetic efforts.[2] Accordingly, these methods are appealing if the calculated results provide effective guidance to medicinal chemistry but represent an inefficient use of time if inaccuracies in the results lead to medicinal chemistry efforts on hits that ultimately fail to be confirmed experimentally.

In contrast, free energy perturbation (FEP)-based methods are low in throughput but produce calculated results quantitatively consistent with experimental results.[3] Success has been demonstrated for calculations of relative differences in lipophilicity, ionization, covalent hydration, and solvation.[4] Moreover, and most important for drug design, the approach is reported to accurately predict relative binding free energies for inhibitors of numerous enzymes,[5] including those considered as potential drug targets.[6] Despite these impressive results, FEP calculations are rarely used in the pharmaceutical industry. Much of the resistance stems from the complexity of the method and its low throughput. FEP calculations are CPU demanding and require the availability of validated molecular mechanics force field parameters to achieve high accuracy. Since most drug candidates contain substructures not fully described by existing parameters, the user must develop and input parameters prior to initiating the calculation. This process is time-consuming and often limited by the absence of relevant experimental data. Accordingly, FEP calculations are inherently difficult to automate and require considerable user expertise and judgment to complete successfully.

Recently, we communicated our successful use of a quantum mechanics (QM)-based FEP method for the calculation of solvation free energy differences.[7] The method used molecular mechanics (MM) for treating the solvent and QM for treating the ligand. Results generated using this method were consistent with those generated using a conventional FEP method. In this communication, we report the results from calculations of relative solvation and binding free energies of AMP analogues complexed with human fructose-1,6-bisphosphatase (FBPase).

FBPase is a rate-controlling enzyme catalyzing the second-to-last step in the gluconeogenesis pathway. Flux through this pathway is abnormally high in type 2 diabetes and largely responsible for the excessive endogenous glucose produced in these patients.[8] Since glucose production contributes significantly to the elevated blood glucose levels associated with diabetes and correspondingly to the disease-related morbidity and mortality, FBPase has long been considered a potential target for treating type 2 diabetes. Discovery of potent FBPase inhibitors, however, has proven to be difficult with little progress made over the past 30 years despite efforts to screen large compound libraries[9] as well as synthesize both substrate and AMP analogues. More recently, we reported the use of a structure-guided drug design strategy that led to the first potent inhibitors of FBPase[10] with demonstration of robust glucose lowering activity in animals with type 2 diabetes.[11]

Calculations using conventional and QM/MD-based FEP methods were performed using procedures previously described.[12] In both cases, the λ-coupling method was used for transforming inhibitor A into inhibitor B (Figure 1)[3] and the thread technique[6,13] for mapping structurally dissimilar molecules. In the conventional method, MM parameters were scaled according to λ and used to calculate MM energies and forces. In the QM/MM-based FEP method, semiempirical QM (AM1) was used to calculate the energies and forces for the ligand while MM was used to calculate the energies and forces of the solvent and protein. The total energy for the system was determined using eq 1 wherein the term $E_{QM/MM}$ represents the interaction energy for an atom i in the MM part of the system and an atom j in the QM part of the system. The free energy change (eq 2) is decomposed into the free energy contribution from the subsystem treated by QM and the free energy contribution from the surroundings, that is, the subsystem not treated by QM (non-QM or NQM).

$$E_{tot} = E_{QM} + E_{MM} + \sum_{M}^{i=1} \sum_{L}^{j=1} E^{ij}_{QM/MM} \quad (1)$$

$$\Delta G_{tot} = \Delta G_{QM} + \Delta G_{NQM} \quad (2)$$

Relative differences in binding free energies were obtained using a two-stage procedure as previously described and 632 ps of molecular dynamic (MD) simulation for each mutation.[12] The calculated results shown in Table 1 suggest that the QM-based FEP method is capable of producing relative binding free energies that trend closer to the experimental results than those produced by the conventional FEP method. The transformations evaluated included replacement of a nitrogen in the heterocyclic purine base with CH (AMP → **1**) as well as replacement of the purine amino group with H (AMP → **2**) or the electron-rich chloro substituent (AMP → **3**). Moreover, relative binding affinities for a transformation associated with a large loss in potency (2000-fold) that involved the replacement of the 5′-oxygen with methylene (AMP → **4**) and a transformation associated with structural changes in both the base and its substituents (AMP to ZMP (**5**)) were also consistent with experimental findings.

The large loss in potency found for phosphonate **4** was of particular importance to our drug design strategy, which was focused on the use of phosphonates as a biologically stable surrogate for the phosphate group and their potential for retaining the six to eight hydrogen bonds formed between the phosphate binding site and the $PO_3^=$.[14] The absence of the 5′-oxygen and therefore the hydrogen

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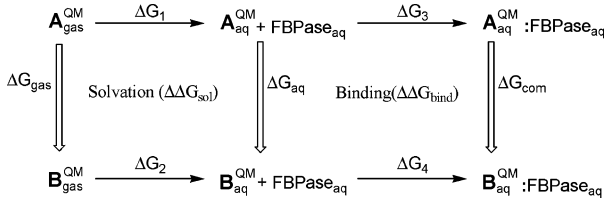


Figure 1. Thermodynamic cycle for computing solvation and binding free energy differences between FBPase inhibitors.

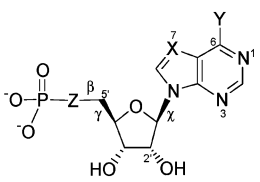
Table 1. Relative Free Energies for AMP Mimics (kJ/mol)

transformation (**A** → **B**)	$\Delta\Delta G_{sol}$[a]	$\Delta\Delta G_{bind}$[a]	$\Delta\Delta G$(con)[b]	$\Delta\Delta G$(exp)[c]
AMP → **1**	4.1 ± 1.5	12.5 ± 1.9	11.9 ± 2.0	13.8
AMP → **2**	16.2 ± 1.6	10.4 ± 2.1	9.9 ± 2.2	11.3
AMP → **3**	19.8 ± 1.7	5.5 ± 2.2	5.1 ± 2.2	5.9
AMP → **4**	6.0 ± 1.8	20.4 ± 2.1	16.9 ± 2.0	>19.0
AMP → **5**	−5.8 ± 2.1	6.5 ± 2.5	7.3 ± 2.6	6.7

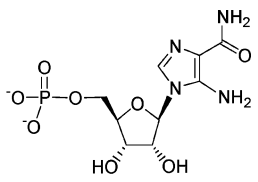
[a] Calculated using AM1 for gradients and ab initio HF/6-31G*/ESP[17] for partial atomic charges. [b] Calculated using a conventional FEP method and HF/6-31G*/ESP for partial atomic charges. [c] Values obtained from experimental data reported in the literature.[12]

bond with the Y113 phenolic hydrogen accounted for part of the decrease in potency[15] but not likely all since hydrogen bonds of this type (OH···O) typically contribute less than 10 kJ/mol toward the binding affinity[16] with the net contribution even less in this case due to the decreased desolvation costs (6 kJ/mol) associated with **4**. To identify the additional factors responsible for the decreased binding affinity, FEP calculations were conducted using the QM/MM method wherein the partial atomic charges of AMP and **4** were updated in each window.

The results showed that this simple transformation led to a significant change in the AMP geometry presumably due to the 0.27 Å increase in the sum of the bond lengths for C−C−P versus C−O−P. Small differences in the torsion angles [$\Delta\chi(C_4-N_9-C_{1'}-O_{1'}) = 6°$; $\Delta\gamma(O_{5'}-C_{5'}-C_{4'}-C_{3'}) = 14°$; $\Delta\beta(P-O_{5'}-C_{5'}-C_{4'}) = 9°$] were observed relative to AMP, resulting in an overall increase in ligand strain of 4.5 kJ/mol. Slight adjustments were also noted in the side chains for the residues in the vicinity of the 5′ position (0.25 Å of rms). Decomposing the relative binding free energy difference into van der Waals and electrostatic energies showed that most of the lost affinity was due to decreases in electrostatic energy (3.2 vs 17.2 kJ/mol), which was attributed primarily to less favorable interactions energies with Y113 (12.6 kJ/mol), K112 (10.1 kJ/mol), and L30 (3.5 kJ/mol). The inability of **4** to retain the favorable binding site interactions exhibited by AMP led to the pursuit of an alternative design strategy, which successfully accessed the phosphate binding site by attaching phosphonates to C8 of the purine base via a small molecular spacer and resulted in the discovery of highly potent and specific FBPase inhibitors.[10]



AMP X = N Y = NH_2 Z = O
1 X = CH Y = NH_2 Z = O
2 X = N Y = H Z = O
3 X = N Y = Cl Z = O
4 X = N Y = NH_2 Z = CH_2



5 (ZMP)

As expected, the QM/MM-based FEP method required more CPU to complete relative to conventional FEP (∼5-fold). This translates for the examples shown to a calculation every 4−5 days using a single processor IBM RS6000. While this represents a significant increase in the total calculation time relative to computational strategies that are less rigorous,[1,2] the benefit is increased accuracy, which in turn is expected to provide additional insights and better guidance for drug design. Moreover, calculation times are expected to decrease significantly in the future with continual advancements in computer hardware along with parallelization of the code to enable simultaneous use of multiple processors. The QM/MM-based FEP method is also expected to avoid the need for time-consuming generation of MM force field parameters and the inaccuracies originating from parameters derived in the absence of experimental data. Elimination of this step simplifies the process and should enable future automation of these calculations. These results are expected to facilitate and promote the use of FEP calculations in the pharmaceutical industry and among scientists focused on the identification and optimization of lead compounds for drug discovery.

Supporting Information Available: Computational details for FEP and MM calculations, partial atomic charges, lists of final atomic coordinates for inhibitors, and complete ref 9. This material is available free of charge via the Internet at http://pubs.acs.org.

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