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Prediction of the potency of inhibitors of adenosine deaminase by QM/MM calculations†

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QM/MM calculations show that the potency of a range of inhibitors of adenosine deaminase correlates with the relative stability of the reaction intermediate at the active site, rather than with the inhibitor binding energy.

Estimates of substrate–protein binding free energies using models based upon empirical force fields[1] are commonly used to quantify pharmacological activity as reflected in measured potency, traditionally reported as either K_i or IC_{50} values. However, inhibition may result not only from the binding of the inhibitor but also from the formation of an intermediate by reaction of the substrate with the enzyme, which then functions as a transition state analogue inhibitor. In this case, as it is not obvious which property of the substrate or intermediate actually correlates with potency, we need to consider not only their binding energies, but also the relative energies of substrate and intermediate at the active site. The latter are not readily obtained from force field calculations alone, but require a quantum mechanical treatment, which must naturally include the effect of the whole of the enzyme. This can be accomplished using quantum mechanical (QM)/molecular mechanical (MM) models[2] which accurately describe both the electronic and steric effects important in enzyme–substrate interactions.

We here describe a study of the inhibition of the enzyme adenosine deaminase (ADA) where evidence for the existence of such a reaction intermediate is afforded by crystallographic studies.[3] This leads to the first example of the use of a QM/MM model to successfully predict a quantitative correlation between the structure of a set of inhibitors and their activity.

ADA is an essential enzyme in the purine metabolic pathway, catalysing the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. Inhibition of ADA can be needed to prevent the deamination of chemotherapeutic agents containing adenine bases and in the treatment of immunological and antiviral conditions.[4] As such it has been the subject of a number of theoretical studies.[5] The catalytic reaction of ADA is considered to involve the formation of an intermediate from the bound substrate following its protonation and nucleophilic attack by an activated water molecule,[3] which is illustrated in Fig. 1 for one of the range of inhibitors that we have studied. We have used a QM/MM model to evaluate the energy difference between the bound intermediate (II) and the corresponding Michaelis complex (I) for five related inhibitors for which IC_{50} values have been measured[6] (Table 1).

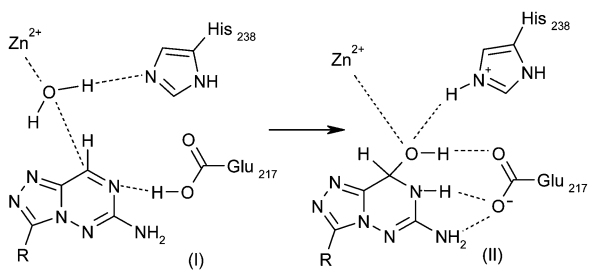
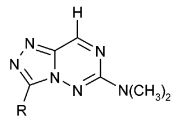
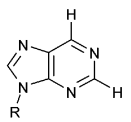
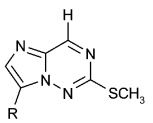
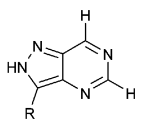
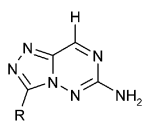


Fig. 1 Michaelis complex (I) and intermediate (II) of inhibitor E, where R is deoxyribose.

The QM/MM calculations follow the strategy previously described.[2a] Initial MM enzyme–substrate models were created, using AMBER,[7] from a crystal structure of a bound intermediate, that of murine ADA complexed to 6-hydroxy-1,6-dihydropurine ribonucleoside (1FKX,[3a] 2.4 Å resolution). This inhibitor was mutated into the five inhibitors (Table 1), and a water molecule was placed in the active site, coordinated to zinc. The resulting structures, which include the crystallographic waters, were solvated in a box of approximately 20000 TIP3P[8] water molecules. Eleven Na^+ ions were added at the positions of largest electrostatic potential to ensure charge neutrality. The force fields for the substrates were constructed from ESP[9] (HF/6-31G(d)) charges for the substrates together with AMBER parameters[10] for related molecules. Parameters describing the interaction of zinc with its ligands were derived from HF/6-31G(d) cluster calculations, van der Waals parameters for zinc being taken from Ryde.[11] The enzyme–substrate models were prepared as follows: energy minimization employing 1000 steps of steepest descent and 9000 steps of conjugate gradient was followed by equilibration using a 20 ps sequence of MD with a 1 fs timestep, the resulting structure being finally minimized. The MD simulation was carried out at 300 K and at constant NPT with a 12 Å non-bonded cut-off, periodic boundary conditions and particle mesh Ewald,[12] the zinc–water distance being restrained to near 2.0 A.

In the QM/MM calculations, these optimized MM enzyme–substrate models were partitioned such that the QM region

Table 1 Five ADA inhibitors and their IC_{50} values. R is deoxyribose

Inhibitor	Structure	IC_{50}/μM
A		180
B		90
C		40
D		5
E		.05

† Electronic supplementary information (ESI) available: Fig. S1–S2 and Table S3. See http://www.rsc.org/suppdata/cc/b3/b305790a/

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included the important catalytic residues, His 238 and Glu 217, terminated at the α-carbon atoms, the substrate and the nucleophilic water molecule (Supporting information Fig. S1†), giving between 40 and 50 atoms in total. The QM region was fully optimized at the QM/MM HF/3-21G level for the Michaelis complex of each inhibitor and corresponding intermediate, followed by energy evaluations at the B3LYP/6-31G(d) level. The gas phase energies of the isolated inhibitor and intermediate were also calculated at the same QM level for comparison with the QM/MM results.

We also evaluated the binding energy of the Michaelis complex as the difference between the substrate–enzyme and substrate–solvent interaction energies, both evaluated at the MM level. Here we take our enzyme–substrate models, and substrate–solvent models similarly constructed, and estimate the net binding energy of each inhibitor using the van der Waals and electrostatic contributions evaluated with the AMBER force field.

In Table 2 we show the results of the three sets of calculations designed to gain insight into the origin of the variation of inhibition for the five molecules studied. We see from the MM binding energies of the inhibitors themselves that there is no correlation between this quantity and their relative potency, for example, B and C are predicted to be more effective than the other three molecules.

Such a lack of correlation between binding energies and potency has also been found for the inhibitor intermediates[6] which suggests that the reactivity of the substrate must be included in any model relating structure to activity. If this is done in the absence of the enzyme by evaluating the energy of the isolated intermediate relative to that of the substrate (gas phase QM energy), then we see that the correlation is poor, in particular A and C are predicted to be more effective than is observed. When the reactive system includes the neighbouring residues (His 238, Glu 217) and the reactive water molecule, as well as the electrostatic and van der Waals interactions with the rest of the enzyme (QM/MM calculation), the energies of the bound intermediate relative to that of the bound substrate for the different inhibitors are now very different from those found in the gas phase (Table 2), and a good correlation between this quantity and potency results (Fig. 2). We do however note that the activity of inhibitor C is somewhat underestimated, suggesting the need for further computational studies.

Analysis of the computational results shows that the relative potency is due to both steric and electronic effects of the enzyme, which in particular mediate the hydrogen bonding interaction of the intermediate with Glu 217 (Supporting information† Fig. S2 and Table S3). A bulky group at the 2-position (in A and C) reduces this stabilizing interaction by lengthening its hydrogen bond with Glu 217, this being crucial to the formation and stabilization of the intermediate. The relative instability of B compared to its isomer D found in the gas phase, arising from the loss of aromaticity in the intermediate, is still present when bound to the enzyme. The enhanced stability of the intermediate formed by E and D, compared to the gas phase can be attributed to a strong hydrogen-bond between Glu 217 and the $–NH_2$ group of the intermediate, and in the latter a lack of the unfavourable steric interaction, present in A and C. It should be noted that optimization of the QM region without the steric constraints imposed by the MM region results in poor correlation with the measured activity.

Table 2 Inhibitor binding energies (MM), and energy of the intermediate relative to the unreacted inhibitor in the gas phase (QM) and at the enzyme active site (QM/MM). The latter includes the energy of all QM atoms and the electrostatic and van der Waals effects of the enzyme environment

Inhibitor	MM binding energy[a]	Gas phase QM energy[a]	QM/MM energy[a]
A	−12.7	0.6	14.8
B	10.3	7.6	12.2
C	4.9	−2.2	15.4
D	−3.4	−0.6	5.0
E	0	0	0

[a] Relative to inhibitor E in kcal mol^{-1}

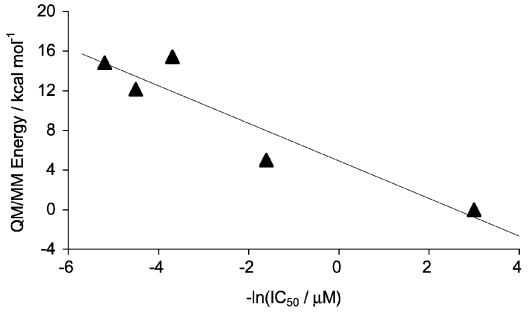

Fig. 2 Plot of energy of intermediate relative to that of the substrate (QM/MM B3LYP/6-31G(d)//HF/3-21G) *versus* $-\ln IC_{50}$. $R^2 = 0.87$. The same trend is observed at the QM/MM HF/3-21G//HF/3-21G level, with an R^2 of 0.88.

The results of these QM/MM calculations show that it is not the binding energies of the substrate or intermediate alone which determine the potency of the different substrates. We find the stability of the bound intermediate compared to that of the substrate to be important for understanding the relative potency of these five inhibitors, suggesting that the equilibrium between these species is responsible for the differing degrees of inhibition.

Although further validation of this approach is clearly required, we suggest that this methodology can be used to predict new inhibitors and points to the potential value of QM/MM calculations in drug discovery, which, to date, remains unexploited.

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